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1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

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3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

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* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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Combined-Modality Treatment of Inoperable Lung Cancer (Iv Immunotherapy, Chemotherapy, and Radiotherapy)[1,2]

Eliezer Robinson,[3,4,*] Nissim Haim,[3] Ruth Segal,[3] Zila Veseley,[3] and Tamar Mekori[3]

Patients with inoperable non-small cell carcinoma of the bronchus were treated by iv methanol extraction residue of bacillus Calmette-Guérin (MER) followed by combination chemotherapy with methotrexate, doxorubicin, cyclophosphamide, and lomustine (MACC). Radiotherapy was added in patients with localized disease. MER was given iv in four weekly courses. The dose ranged between 0.1 and 0.8 mg/m^2/course and was determined according to the skin reactivity to MER. None of the 19 patients treated with iv MER had an objective tumor response during immunotherapy. Five of 16 evaluable patients receiving MACC (31%) achieved partial response. The overall median survival for all 17 patients treated was 11 months according to the protocol. During immunotherapy the following side effects were observed: all patients had fever and chills and most had malaise, nausea and/or vomiting, and headache. Transient abnormalities of liver function tests were found in six patients, and in one patient roentgenographic changes suggestive of lung granulomas were seen. The side effects were more severe during the first course than in the further treatments. Side effects of the MACC combination were comparable to those observed in previously reported studies. Immunological monitoring performed during immunotherapy revealed that iv MER had an unfavorable effect on the immune system. Following four courses there was a decrease in the in vivo skin reactivity to MER and to five recall antigens. Twenty-four hours after the initiation of iv MER there was a tendency for decrease in the lymphoproliferation to phytohemagglutinin, an increase in the indices of the adherent cell suppressor system and the prostaglandin-related suppressor system, and an increase in the percentage of adherent mononuclear cells. No consistent changes in serum lysozyme were found. A transient increase in the total number of peripheral blood leukocytes and a decrease in the number of lymphocytes were noticed 24 hours after iv MER. Taking into account the results of the immunological studies and the overall therapeutic results, we do not feel justified in continuing to use this combined modality treatment. [Cancer Treat Rep 69:251–258, 1985]

Immunotherapy with bacillus Calmette-Guérin (BCG) has been reported to stimulate the immune response and to improve survival in patients with various types of malignant tumors (1–3). The methanol extraction residue of BCG (MER) is a potent immunostimulant and has certain advantages over BCG by being nonviable and stable (4,5). In previous reports from our center it was found that intradermal MER stimulates the cell-mediated immune responses in patients with inoperable lung cancer (6,7). However, survival was not significantly improved by the intradermal injection of MER (6). Iv MER administration has been shown to be tolerable and to have an immunostimulant effect (8–10). It has the advantage of avoiding skin reactions.

In an attempt to improve the therapeutic results in advanced non-small cell carcinoma of the bronchus, we added iv MER to a standard therapy for this disease. Following immunotherapy, patients were treated with a combination of methotrexate, doxorubicin, cyclophosphamide, and lomustine (CCNU) (MACC) (11–13); radiotherapy was also given to patients with localized disease.

This paper describes the results of the immune monitoring performed during the iv administration of MER as well as the overall results of this phase II study.

[1]Received Nov 11, 1983; revised Aug 7, 1984; accepted Aug 16, 1984.

[2]Supported by a grant from the National Council for Research and Development, Israel, and by the D.K.F.Z., Heidelberg, W Germany.

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MATERIALS AND METHODS

Patients

Twenty-two patients with a histological diagnosis of non-small cell carcinoma of the lung were entered in this study between October 1980 and December 1981. The criteria for patient selection were as follows: (*a*) age < 75 years old; (*b*) performance status of 7 or more (Karnofsky scale); (*c*) absence of liver or brain metastases; (*d*) adequate liver and renal functions (bilirubin < 1.4 mg/dl, urea < 50 mg/dl, and creatinine < 1.5 mg/dl); and (e) no evidence of active heart disease contraindicating doxorubicin administration. Each patient gave informed consent. Nineteen of the 22 patients completed four series of MER treatment (two stopped treatment due to intercurrent disease and one died of unrelated cause). Chemotherapy was administered to 17 of the 19 patients (one patient refused chemotherapy and in one it was contraindicated due to acute ischemic heart disease). Ten patients with localized disease received radiotherapy.

Immunotherapy

MER-BCG (Phipps strain supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) was administered iv in four weekly courses. Dose was determined according to a skin test to 0.1 ml of five dilutions of the original MER solution (1:1, 1:10, 1:100, 1:1000, and 1:10,000) dose prior to immunotherapy. The iv MER dose was determined according to the skin test which led to a skin induration of 5–10 mm without ulceration. The treatment doses (and the corresponding skin test dilutions) were 1.25 mg/m^2 (1:1), 0.8 mg/m^2 (1:10), 0.4 mg/m^2 (1:100), 0.2 mg/m^2 (1:1000), and 0.1 mg/m^2 (1:10,000), respectively. MER was infused over 4 hours iv in 150 ml of normal saline. Pulse, blood pressure, and temperature were monitored at least every 4 hours or until the fever disappeared. Fever was defined as elevation of temperature to ≥ 37.6°C.

Chemotherapy

MACC was started 7 days after the fourth course of iv MER and consisted of iv methotrexate (40 mg/m^2), doxorubicin (40 mg/m^2), cyclophosphamide (400 mg/m^2), and CCNU (30 mg/m^2) given orally on the same day. Courses were repeated every 3 weeks. MACC was stopped during radiotherapy and was continued 2–4 weeks after its termination. Other details of drug administration and clinical evaluation as well as follow-up of the patients during MACC were done as previously described (12). MACC was discontinued at evidence of tumor progression (at least three series were required to determine MACC failure).

Radiotherapy

Radiotherapy was given 3 weeks after the third course of MACC in patients with limited disease (ie, tumor confined to one lung, including mediastinal and/or ipsilateral supraclavicular nodes) and with adequate respiratory function. Four thousand to 4600 rads in fractions of 200 rads given five times a week were administered through two opposing fields by 8-MeV linear accelerator to the primary tumor and mediastinum. Additional 1000–1500 rads were administered to the primary tumor through reduced fields. The supraclavicular regions were irradiated only when these nodes were clinically positive.

Clinical and Laboratory Investigations

Chest x-ray was performed once a week during MER administration and afterwards every 3–4 weeks. Liver and bone scans were performed only if indicated. Prior to MER therapy, the following laboratory tests were routinely performed: cbc, SGOT, alkaline phosphatase (AP), bilirubin, blood proteins, urea creatinine, uric acid, calcium, phosphorus, and other electrolytes. Cbc, SGOT, AP, and bilirubin were repeated serially before and 1 day after each course of MER.

In Vivo Immunological Studies

Delayed hypersensitivity skin testing (DHS) was evaluated by skin test to MER (using the five dilutions previously described) and to the following five recall antigens: purified protein derivative (PPD) (Ministry of Health, 2 tuberculin units), streptokinase-streptodornase (Lederle, 40/10), candidin (0.1%), trichophytin (0.1%), and mixed bacteria (0.1% of a mixed suspension of staphylococcus and streptococcus); MER was injected intradermally at the lateral aspect of the arms. The average of two diameters of induration was recorded after 7 days. The five recall antigens were injected on the forearms, and the reaction was recorded after 48 hours. The results were expressed as the mean cumulative induration to all five antigens.

In Vitro Immunological Studies

Mononuclear cell separations.—Peripheral blood mononuclear cells (MNL) were separated from freshly drawn heparinized blood by centrifugation (1200 rpm) over Ficoll-Urografin 60% gradient (Pharmacia Fine Chemicals AB, Uppsala, Sweden) for 30 minutes. The cells were washed three times with normal saline and resuspended in medium M-199 supplemented by 100 units/ml of penicillin and 100 μg/ml of streptomycin. Cell viability was determined by eosin.

Cultures technique.—MNL were cultured in flat-bottomed microtiter plates (Costar). Each culture contained

10^5 MNL in 0.2 ml of M-199 supplemented with 20% heat-inactivated pooled human AB serum. Cultures were set up in triplicates and stimulated with 50 μg/well of phytohemagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England). They were incubated for 3 days at 37°C in a humidified 5% CO_2 atmosphere. Eighteen hours before harvesting, 0.2 μCi of ^{3}H-thymidine was added to each culture. Cultures were harvested by a multiple automatic-sample Israeli-made harvester. Incorporation of ^{3}H-thymidine into DNA was measured by a Packard liquid scintillation counter and expressed in counts per minute.

Adherent cell suppressor system (ACSS).—Five milliliters of cell suspension containing 10.10^6 cells in M-199 and 20% AB serum was incubated at 37°C for 1 hour in plastic Petri dishes (Nunc, 5 cm in diameter). After incubation, the nonadherent cells were collected, centrifuged (1200 rpm for 7 minutes), and resuspended in M-199. Cultures of 10^5 cells were set up and stimulated with PHA (Wellcome Research Laboratories) at a dose of 50 μg/well before and after removal of adherent cells. The ACSS index was calculated as follows:

$$\text{ACSS index} = \frac{\text{lymphocyte proliferation (LP) by PHA after removal of adherent cells}}{\text{LP by PHA before removal of adherent cells}}.$$

The percentage of adherent cells was also calculated.

Prostaglandin-related suppressor cell system (PgSS).—Indomethacin (Sigma Chemical Co) dissolved in 95% ethanol diluted in M-199 was added to MNL cultures in final concentration of 1 μg/ml. The PgSS index was expressed as follows:

$$\text{PgSS index} = \frac{\text{LP by PHA with indomethacin}}{\text{LP by PHA without indomethacin}}.$$

Serum lysozyme levels were determined using the lysoplating assay as described by Osserman and Lawler (14).

Statistical methods.—The significance of quantitative difference between various groups was determined in accordance with the Mann-Whitney rank-sum test. Differences in the results of serial skin testing were evaluated with the aid of the t pairs test.

RESULTS

MER Therapy

Major patient characteristics are shown in table 1. The dose for each course given to the patients ranged from 0.1 to 0.8 mg/m^2 (mean, 0.27). During immunotherapy, tumor progression was noticed in three of the 19 patients (16%) and in the remaining patients the disease was stable. Side effects were mild to moderate, and in none of the 19 patients was it necessary to stop MER or decrease the dose. As a rule, adverse reactions were more severe during the first course; in subsequent courses, adverse reactions were more tolerable. Side effects recorded in the 19 patients who completed four courses of MER are summarized in table 2. During the first course, all patients had fever and chills, which started 2–10 hours after initiation of therapy. The duration of fever during the first course (2.75 ± 0.96 days; mean ± SD) was significantly longer than during the fourth course (0.93 ± 0.58 days; $P < 0.001$). The mean maximal temperature reached during the first course (39.25°C ± 0.77°C; mean ± SD) was also significantly higher than that reached during the last course (38.22°C ± 0.73°C; $P < 0.01$).

Three patients (16%) did not have temperature elevation during the fourth course. Nausea, vomiting, and headaches were usually mild and responded to standard symptomatic treatment. An increase in the pulse rate corresponded to elevation of temperature. There were no significant changes in blood pressure.

A consistent pattern of changes in liver function tests during MER courses was observed (fig 1). Immunotherapy caused an increase in serum AP levels, which was maximal 7 days after the first course, and a corresponding increase in SGOT levels, which was maximal 1 day after the second course. Four of the six patients who had

TABLE 1.—Clinical characteristics of 19 patients treated with 4 courses of iv MER

Characteristic	No. of patients (%)
Mean age in yrs (range): 63.6 (44–75)	
Sex	
Female	2 (11)
Male	17 (89)
Histology	
Squamous cell carcinoma	10 (53)
Adenocarcinoma	7 (37)
Large cell carcinoma	1 (5)
Adenocarcinoma and squamous cell carcinoma	1 (5)
Stage	
Limited disease	16 (84)
Extensive disease	3 (16)
Median Karnofsky performance status (range): 9 (7–10)	

TABLE 2.—Side effects of iv MER therapy (19 patients)

Side effect	No. of patients (%)
Fever and chills	19 (100)
Malaise	16 (84)
Nausea and/or vomiting	13 (68)
Headache	10 (53)
Transient abnormalities* of liver function test	6 (32)
Roentgenographic changes suggestive of lung granulomas	1 (5)

* Increase in AP or in SGOT levels of at least twice the upper normal limit.

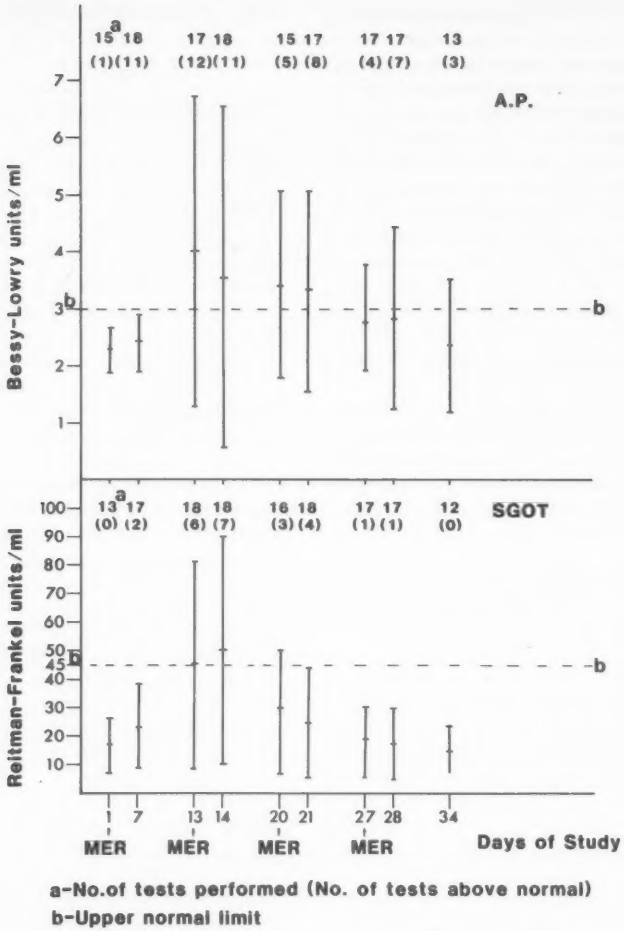

FIGURE 1.—Effect of iv MER on serum AP and SGOT (mean ± SD).

an increase of AP and/or SGOT levels of at least twice the upper limit of the normal range also had transient hyperbilirubinemia (range, 1.4–4.0 mg/dl). Liver function tests returned to pretreatment levels after MER was stopped. No significant changes in the other blood chemistry tests were observed.

One patient developed bilateral pulmonary infiltrates, which were not accompanied by respiratory distress and which disappeared when MER therapy was stopped.

Chemotherapy-Radiotherapy Results

Sixteen of the 17 patients treated with MACC were evaluable. Five patients (31%) had partial response. All patients have already died. The median survival times for responders, nonresponders, and the entire group of 17 patients were 17, 9, and 11 months, respectively. Toxicity of MACC was moderate and is summarized in table 3. There were no drug-related deaths. No major side effects due to radiotherapy were observed.

In Vivo Immune Monitoring

Skin testing was performed 1 week before the initiation of immunotherapy and 1 week after the last course of iv MER. Table 4 shows that skin reactivity to PPD, the cumulative skin sensitivity to the five recall antigens, and the DHS to MER decreased following immunotherapy. The change in the size of reaction to PPD and in the cumulative antigens was statistically significant ($P < 0.01$).

TABLE 3.—Toxicity observed in patients treated with MACC

Adverse reaction	No. of patients/ No. evaluated (%)
Stomatitis	7/17 (41)
Cardiomyopathy (due to doxorubicin)	1/17 (6)
Fever and leukopenia	5/17 (29)
Acute gastrointestinal bleeding and thrombocytopenia	1/17 (6)
Wbc count (cells/mm^3)	
< 4000	12/15 (80)
< 2000	5/15 (33)
Platelet count (cells/mm^3)	
< 100,000	5/15 (33)
< 50,000	2/15 (13)

In Vitro Immune Monitoring

The in vitro assays were performed at the following intervals: 1 week before the first course of iv MER (Day 0), 1 week after the fourth course, immediately before the initiation of each of the four courses, and 24 hours after each iv MER. A relatively high variability in the results was found at each time interval. The results of LP to PHA, of the ACSS and PgSS indices, and of the percentage of adherent cells (assessed in the ACSS) are shown in table 5 and in figure 2. A statistically significant decrease in LP to PHA was observed 24 hours after each of the first three courses of MER. Six days later there was a partial recovery. The LP values after 6 days did not reach the pretherapy values.

Suppression indices tested 24 hours after iv MER tended to be higher than the corresponding indices tested immediately before MER. This increase was statistically significant after the third course of both ACSS and PgSS and after the first course of ACSS. An index > 1.0 was defined as suppression. Data not shown in table 5 indicate that the percentage of patients showing suppression (ACSS) was increased from 25% before the first course of iv MER to 56.25% 24 hours later ($P < 0.05$).

One day after each course of MER there was an increase in the percentage of adherent cells. This change was statistically significant after the first and third courses.

The results of the peripheral wbc counts and of serum lysozyme levels are presented in table 6. There was a statistically significant increase in the total number of wbcs 24 hours following the first and second MER courses. The number of lymphocytes was significantly decreased 24 hours after each of the four courses of MER. However, following another 6 days there was a complete recovery in the lymphocytes. No consistent changes in serum lysozyme levels before and after MER-courses were observed.

DISCUSSION

The few reported studies of MER administration (8-10,15-17) have indicated that this modality of immunotherapy could be given with acceptable toxicity, with doses varying widely from 0.02 to 1.25 mg/m^2/course. Vogl et al (10) recommended that the dose for each course should not exceed 0.32 mg/m^2, while Maroun et al (9) suggested a dose of 0.1 mg/m^2 to patients with prior treatment with BCG or with positive PPD skin test and a dose of 0.5 mg/m^2 for other patients. The results obtained from this phase II study show that an iv MER dose could reliably be determined according to the skin reactivity to MER. Side effects during the first course were mild to moderate and not related to the MER treatment dose. There was no evidence of cumulative toxicity, which is in accordance with the report of Maroun et al (9). Furthermore, there were fewer side effects in subsequent courses. At present, we have no explanation for these findings. However, in view of the reduced toxicity in subsequent courses, dose escalation could be tested in future studies.

Reported adverse effects from iv MER include fever and chills, nausea and/or vomiting, tachycardia, headache, acute urticaria and other skin reactions, moderate decreases in pulmonary vital capacity, and miliary-type pulmonary parenchymal changes (15). The latter are considered as one of the most serious complications and occurred in only one of 19 patients compared to eight of 64 patients in other series (15). After the treatment was stopped, chest x-ray was normal. While Maroun et al (9)

TABLE 4.—Skin reactivity of patients tested before and after 4 courses of iv MER

	No. of patients	Before treatment (mm, mean ± SD)	After MER (mm, mean ± SD)	Statistical significance (t pairs test)
Skin test to PPD	15	12.87 ± 7.62	5.6 ± 7.18	$P < 0.01$
Cumulative values of skin test to 5 antigens	15	7.47 ± 5.23	2.77 ± 2.4	$P < 0.01$
MER	12	21.37 ± 8.16	15.58 ± 8.27	NS*

* Not significant.

TABLE 5.—LP to PHA, IS, and % of adherent cells

Day of study	LP: No. of patients	LP (cpm) (mean ± SD)	*P* value	IS ACSS: No. of patients	IS ACSS (mean ± SD)	*P* value	IS PGSS: No. of patients	IS PGSS (mean ± SD)	*P* value	Adherent cells: No. of patients	% adherent cells (mean ± SD)	*P* value
0	19	33,256 ± 18,076		19	0.86 ± 0.4		17	1.35 ± 0.56		17	29.77 ± 26.2	
7*	19	40,252 ± 14,215	< 0.001	19	0.83 ± 0.27	< 0.05	15	1.12 ± 0.48		19	38.47 ± 21.9	< 0.01
8	16	22,017 ± 15,324		16	1.17 ± 0.55		14	1.3 ± 0.64		17	45.94 ± 28.32	
14*	19	32,392 ± 19,462	< 0.02	19	1.23 ± 0.82		14	1.36 ± 0.5		19	34.05 ± 23.62	
15	18	17,763 ± 12,952		18	1.2 ± 0.54		14	1.47 ± 0.5		18	39.22 ± 24.25	
21*	18	30,554 ± 15,300	< 0.02	17	1.08 ± 0.48	< 0.05	16	1.3 ± 0.5	< 0.05	18	28.94 ± 19.30	< 0.02
22	13	17,227 ± 12,377		15	1.49 ± 0.66		15	1.57 ± 0.9		14	49.93 ± 25.46	
28*	19	24,139 ± 12,636		19	0.95 ± 0.6		18	1.32 ± 0.5		18	30.55 ± 23.53	
29	16	32,280 ± 27,232		16	1.12 ± 0.6		16	1.1 ± 0.25		15	36.33 ± 30.7	
35	15	27,727 ± 11,787		15	0.92 ± 0.48		15	1.6 ± 0.34		15	33.86 ± 21.19	

* Day of MER administration.

TABLE 6.—Wbc counts and serum lysozymes before and during MER administration

Day of study	Leukocyte count: No. of patients	No. of leukocytes (mean ± SD)	*P* value	Lymphocyte count: No. of patients	No. of lymphocytes (mean ± SD)	*P* value	Monocyte count: No. of patients	No. of monocytes (mean ± SD)	*P* value*	Serum lysozymes: No. of patients	Serum lysozymes (mean ± SD)
0	19	7852 ± 2596		17	1819 ± 802		13	188 ± 130	NS	17	7.2 ± 2.6
7	19	7103 ± 2069	< 0.02	18	1882 ± 804	< 0.02	12	197 ± 121	NS	16	7.7 ± 2.8
8	16	8869 ± 1874		17	1285 ± 498		13	285 ± 101	NS	16	7.7 ± 3.2
14†	19	7415 ± 2243	< 0.1	18	1944 ± 923	< 0.05	15	182 ± 122	NS	14	8.9 ± 3.5
15	18	9283 ± 3594		17	1270 ± 697		10	160 ± 81	NS	14	8.5 ± 3.1
21†	18	8894 ± 2382		17	1967 ± 849	< 0.01	13	258 ± 184	NS	13	8.6 ± 2.9
22	13	7973 ± 3514		12	1011 ± 676		10	233 ± 222	NS	12	10.6 ± 2.8
28†	19	8134 ± 2347		17	2173 ± 1057	< 0.001	10	200 ± 107	NS	12	8.2 ± 2.5
29	16	8100 ± 2224		14	1389 ± 708		8	147 ± 63	NS	13	8.2 ± 3.2
35	15	8006 ± 3355		14	2043 ± 1491		12	190 ± 95	NS	10	10.3 ± 5.4

* NS = not significant.
†Day of MER administration.

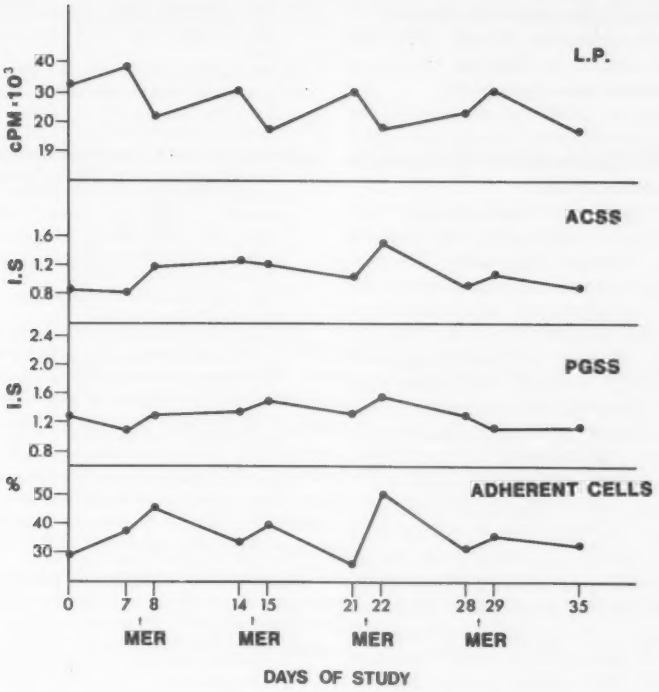

FIGURE 2.—Effect of therapy on LP by PHA, index of suppression (IS), and % of adherent cells.

did not find any consistent changes in liver function tests performed serially during iv MER, Vogl et al (10) have reported that nine of 12 patients who received iv MER at doses of ≥ 0.08 mg/m^2 had an elevation of serum AP of 25%, and one had a 3.5-fold increase in SGOT as well. In the present study, we have found a special pattern of changes in liver function tests (ie, maximal elevation in AP and/or SGOT levels after the first two courses of iv MER and return to normal levels within 1 week after the fourth course). Four of 19 patients had transient hyperbilirubinemia. These findings indicate that the above-mentioned metabolic changes were due to hepatotoxic effect of MER and not due to liver or bone metastases. Our data also show that the changes are reversible despite the continuation of the MER therapy. Granuloma formation in the liver due to iv MER has been demonstrated in dogs (17) and may explain the mild hepatotoxic effect of iv MER.

The particular timing for MER administration used in this study was selected for two reasons. First, it has been proven in animal studies that a time interval between immunotherapy is an important condition for the success of immunotherapy (18,19). Second, the administration of MER before MACC made it possible to evaluate the MER effects more precisely.

Several groups of investigators have found that MER, when given intradermally, acts as an immunostimulant (20). However, only few data are available pertaining to the effect of iv MER on the immune system. In two previously reported studies (8,10), no consistent changes in skin reactivity to recall antigens were found before and after iv MER. In a recent study, Hersh et al (16) have evaluated the following host defense parameters: the number of adherent macrophage precursors, serum lysozyme, and antibody-dependent cell-mediated cytotoxicity. During weekly MER therapy all of these parameters rose significantly. These investigators believe the above methods can quantitatively reflect activation of monocytes and killer cells by immunotherapy.

The data obtained from the present study provide no evidence that iv MER stimulates the immune system. On the contrary, immunotherapy was associated with an unfavorable effect on cell-mediated immunity. Following four courses of iv MER there was a depression in the in vivo skin reactivity to MER and to recall antigens. In vitro LP to PHA decreased during iv MER therapy, and this decrease was especially evident 24 hours following the first three courses. As indicated by the results obtained for the ACSS and PgSS assays, the decrease in the lymphoproliferative response of lymphocytes could have

resulted from an increase in the number of adherent cell suppressor cells and the activation of both ACSS and PgSS caused by MER. Contrary to Hersh et al (16), we found no obvious increase in serum lysozymes.

While other investigators (9,10) did not observe any consistent changes in the wbc count, we noticed a transient increase in the total number of wbcs and a decrease in the lymphocytes following iv MER.

McKneally et al (21) found that immunotherapy with BCG by the intrapleural route improved the survival rate of a subset of patients with stage I lung cancer. However, the survival of patients with more advanced disease was not improved by BCG immunotherapy. In a previous report from our center the survival of patients with advanced lung cancer was also not significantly improved by intradermal MER (6). These results were, indeed, consistent with animal studies which demonstrated that immunotherapy is effective only for a limited tumor burden (22). The use of iv MER in the treatment of lung cancer has not been reported to the best of our knowledge.

Since the current study was nonrandomized, it is difficult to determine whether iv MER had any influence on the overall therapeutic results. Nevertheless, the results obtained do not point to any favorable effect of MER. None of the patients achieved an objective tumor response to immunotherapy, and the subsequent response rate to the MACC combination (31%) is within the range reported in the literature (12%-44%) (11-13). Likewise, comparison of the side effects of MACC in this series to those reported previously (11-13) does not indicate that iv MER reduced drug toxicity significantly. The apparent lack of effectiveness of iv MER is consistent with the results of the immune monitoring observed in this study.

In conclusion, we found that iv MER has an unfavorable effect on the immune system in addition to no antitumor effect. It also did not seem to modify the therapeutic effect of chemotherapy and its toxicity. We therefore do not feel justified in continuing the present protocol.

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Etoposide and Very High Dose Cisplatin: Salvage Therapy for Patients With Advanced Germ Cell Neoplasms[1,2]

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Twelve patients with refractory germ cell tumors were treated with etoposide (VP16) (100 g/m²/day × 5) and very high dose cisplatin (VHD-CDDP) (40 mg/m²/day × 5) every 28 days. All patients had progressed or relapsed after therapy with vinblastine (0.15 mg/kg/day × 2), CDDP (20 mg/m²/day × 5), and bleomycin (30 units/week). Thirty-three cycles of VP16 plus VHD-CDDP were administered. Five patients achieved complete response (two with chemotherapy only, two with chemotherapy + surgery, and one with chemotherapy + irradiation), five achieved partial response (two continue therapy), and two had disease progression. Only one patient achieved prolonged complete response (24+ months). Myelosuppression (median wbc count nadir, 1200/mm³; median platelet count nadir, 18,500/mm³), neurotoxicity (five patients with diminished hearing and six with substantial peripheral neuropathy), and hypomagnesemia (21 of 22 courses; magnesium, < 1.5 mg/dl) were the dominant toxic effects. No important nephrotoxicity was seen (median maximum creatinine, 1.2 mg/dl; range, 0.8–2.2). VP16 plus VHD-CDDP is safe and tolerable and may provide a non-cross-resistant alternative to vinblastine, CDDP, and bleomycin. [Cancer Treat Rep 69:259–261, 1985]

While most patients with testis cancer who are appropriately managed from the time of presentation should be cured, 10%–20% of patients die of disease which is refractory to treatment with cisplatin (CDDP)-based combination chemotherapy. Improved therapy is needed for patients with poor prognosis and for patients failing to achieve a durable remission following initial therapy.

Ozols et al (1,2) have recently demonstrated that the administration of very high dose CDDP (VHD-CDDP) (40 mg/m²/day × 5) is safe and effective. The steep dose-response curve for CDDP in in vitro systems and the clinically observed dose-response relationships suggest that such a dose escalation may overcome resistance to standard-dose (20 mg/m²/day × 5) CDDP.

This report describes our initial experience with a "salvage regimen" for patients with testis cancer employing a potentially non-cross-resistant regimen of etoposide (VP16) and VHD-CDDP.

MATERIALS AND METHODS

Twelve patients were treated with VP16 at a dose of 100 mg/m²/day × 5 and CDDP at a dose of 40 mg/m²/day × 5. CDDP was infused over a 1-hour period in 250 ml of 3% sodium chloride. Twelve hours before CDDP administration, hydration with normal saline with added potassium chloride (20 mEq/L) was begun (250 mg/hour) and continued for 5 days. Furosemide (20 mg) was administered daily before CDDP. VP16 was administered in 500 ml of normal saline over a 1-hour period. Treatment cycles were repeated every 28 days.

All patients had previously received VBP (vinblastine, 0.15 mg/kg/day × 2; bleomycin, 30 units/week; and CDDP, 20 mg/m²/day × 5) every 21–28 days. Eleven patients had received three or more cycles of VBP, while one patient had received one treatment.

In three patients, short-duration complete remission (CR) had been attained with prior VBP therapy (3, 3, and 5 months), while in the remaining nine patients, partial response (PR) was the best response obtained following VBP. Ten patients had typical germ cell tumors (seven testicular, three extragonadal, eight nonseminoma, and two seminoma). In two patients, undifferentiated midline cancers histologically compatible with germ cell tumors

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were treated. All patients had clinically normal hearing and normal blood cell counts. Serum creatinine was < 1.8 mg/dl in each patient. The stages of disease were IIIA (one patient), $IIIB_2$ (two), $IIIB_3$ (three), and $IIIB_5$ (six) (3).

RESULTS

Thirty-three cycles of VP16 plus VHD-CDDP were administered. The following distribution of cycles administered was seen: one (two patients); two (two); three (six); four (one); and five (one).

Response

Two patients with stage $IIIB_2$ disease and increased β-human chorionic gonadotropin obtained CR (normal physical examination, radiographic studies, and normal markers for > 1 month). Both patients had previously achieved short-duration CR with VBP. Duration of CR was short (2 and 3 months). Eight patients had PR (> 50% decrease in size of tumor masses and > 90% decrease in previously increased markers lasting > 1 month). In three patients, CR was achieved by treatment of residual masses (two patients with viable cancer in resected specimens had resection and one patient had radiation therapy to residual para-aortic seminoma). Of these latter three patients, one who achieved CR with resection of residual disease remains in CR 24 months after last therapy; two relapsed at 2 and 4 months. Neither of the two patients thought to have occult germ cell tumors responded; both died within 4 months of VP16 plus VHD-CDDP. Three patients continue to receive therapy. Six patients are alive: one without disease and five with disease receiving therapy.

Toxicity

Median nadir wbc count was 1200/mm^3 (range, 100–3700) for 33 cycles of therapy. Fever and leukopenia occurred following 14 of 33 cycles. Median nadir platelet count was 18,500/mm^3 (range, 2000–150,000). Platelet counts < 20,000/mm^3 occurred during 16 of 33 cycles. No clinically significant bleeding occurred. Day 14 was the median day of maximum myelosuppression. Recovery of granulocyte and platelet counts was evident by Day 21 in all patients.

Clinically important renal function impairment was not seen. Median maximum serum creatinine was 1.2 mg/dl (range, 0.8–2.2). During only two cycles of therapy did the serum creatinine rise to 2.0 mg/dl or more (2.0 and 2.2 mg/dl). Serum creatinine returned to pretreatment values in all patients within 21 days of therapy. Severe hypomagnesemia was common; following 22 cycles of therapy, serial serum magnesium data were available. Hypomagnesemia (< 1.5 mg/dl) occurred in 21 cycles. In nine of 22 cycles, serum magnesium values of < 1.0 mg/dl were documented. Symptomatic hypocalcemia and hypomagnesemia were not seen. Peripheral neuropathy which impaired fine hand movements and gait occurred in six patients. Five patients developed clinically evident hearing loss.

DISCUSSION

Ozols was the first investigator to demonstrate that the regimen employed in this report allows the administration of CDDP at twice the usually employed dose without nephrotoxicity. The present study confirms that VHD-CDDP may be administered without clinically significant nephrotoxicity.

The degree of myelosuppression seen in our patients was impressive. Williams et al (3) reported a 16% incidence of platelet counts < 25,000/mm^3 in 45 patients treated with VP16 (100 mg/m^2/day × 5), CDDP (20 mg/m^2/day × 5), doxorubicin (40 mg/m^2 × 1), and bleomycin. In a second series, the median wbc count nadir was 1100/mm^3 and the median platelet count nadir was 60,000/mm^3 in 33 patients treated with a similar regimen with slightly lower doses of VP16 (300 mg/m^2) (4). Fitzharris et al (5) described 25 patients treated with VP16 as a single agent with two different doses of VP16 (either 120 mg/m^2/day or 100 mg/m^2/day × 5). Eleven patients never had a wbc count < 3000/mm^3 or a platelet count < 100,000/mm^3. Hearing loss and peripheral neuropathy were important findings in Ozols' patients and in our series. It appears that bone marrow and neurotoxicity are the limiting side effects of CDDP administered at 40 mg/m^2/day × 5.

The two largest series describing the use of VP16 as a single agent for salvage treatment of advanced germ cell cancer noted response rates of 45% and 20%, respectively (5,6). CRs were noted in 12.5% of 24 patients in Fitzharris' trial and none of 30 in Varini's trial. Williams et al (7) have recently described the Indiana/Southeastern Oncology Group experience with VP16-CDDP salvage therapy for patients failing or relapsing after VBP therapy. The CR rate of chemotherapy or chemotherapy plus surgery in this series was 44%, and only 24% of patients appeared to have a durable CR. The great majority of patients achieving CR after VP16-CDDP previously achieved CR with VBP. Potentially curative salvage therapy is available for a distinct minority of patients failing therapy with VBP.

In view of the clinical and preclinical data supporting a steep dose-response curve for CDDP and the demonstration of the feasibility of VHD-CDDP therapy, it seems appropriate to consider the use of salvage therapy which *may* be non-cross-resistant for patients with advanced germ cell cancer. However, our data and those of others indicate that patients who fail to achieve a CR with VBP are rarely cured. With improved ability to delineate patients who have limited potential for CR with VBP, con-

sideration should be given to employing VP16 plus VHD-CDDP as part of initial therapy for patients with poor prognosis.

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Treatment of Renal Cell Carcinoma With α(Human Leukocyte) Interferon and Vinblastine in Combination: A Phase I–II Trial[1,2,3]

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Twenty-four patients with measurable metastatic renal cell carcinoma were treated in a phase I–II trial with α(human leukocyte) interferon (αIFN) and vinblastine (VBL) in combination. Patients received 3 × 10^6 IU/day of αIFN im 5 days/week and VBL iv weekly (at a starting dose of 0.15 mg/kg), with doses modified for toxicity. All patients were evaluable for toxicity; 23 patients were evaluable for response. An objective response rate of 13% was observed (three partial responses). An additional 22% of patients had minimal responses (five patients). The occurrence of nausea, vomiting, thrombocytopenia, hepatic dysfunction, and fever was comparable to that seen in previous studies of αIFN alone. Granulocytopenia, neurotoxicity, and malaise, however, occurred with increased frequency and severity. αIFN and VBL administered in combination in this dose schedule demonstrated activity similar to but toxicity greater than that seen in previous trials of αIFN alone.
[Cancer Treat Rep 69:263–267, 1985]

The antineoplastic activity of the alpha interferons (IFN) against metastatic renal cell carcinoma is well-documented. Numerous studies utilizing different preparations of IFN (human leukocyte, human lymphoblastoid, and α-recombinant leukocyte) have demonstrated response rates (complete and partial) varying between 7% and 28% (1–5). We recently reported the University of California at Los Angeles experience, in which 16.5% of 43 evaluable patients treated with human leukocyte IFN achieved an objective (partial and complete) response rate (6).

αIFN in combination with other IFN preparations or "classical" chemotherapy has not as yet received substantial clinical evaluation. αIFN recently has been demonstrated to have significant synergistic activity in combination with vinblastine (VBL) in the human tumor stem cell assay (7). Hrushesky and Murphy (8), reviewing the experience with 33 chemotherapeutic agents in advanced renal cell cancer, found that VBL had the highest reported objective response rate (25%) and was superior to any other single agent.

In an attempt to increase the therapeutic efficacy of αIFN and to evaluate potential additive toxic effects, we have conducted a phase I–II study of αIFN in combination with VBL as therapy for advanced renal cell carcinoma. This paper presents the results of that study as well as an update of our original αIFN trial.

MATERIALS AND METHODS

The αIFN, prepared from buffy coats, was obtained from the New York Blood Center. The IFN was prepared in 0.01 M sodium phosphate buffer (pH 7.0) and was 0.1%–1% pure. Four lots (126, 127, 128, and 130) were used during this study. The specific activity ranged from 2.0 to 3.2 × 10^6 IU/mg of protein. Sendai virus and hepatitis surface antigen were absent from the lots tested.

Patient Study Population

Between May and October 1983, 24 patients with metastatic renal cell carcinoma were entered in this

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study. Patient eligibility required histologically confirmed renal cell carcinoma with advanced disease, either manifest as distant metastasis or progressive local disease not controlled or controllable by surgery. Patients were required to have growing measurable disease. For hepatic lesions, this was defined as a liver defect ⩾ 5 cm in diameter by liver-spleen scan or computed tomography or malignant hepatomegaly extending ⩾ 5 cm below the costal margin. Abdominal disease measured by computed tomography also had to be ⩾ 5 cm in diameter. Bone lesions, effusions, and tumor markers (eg, carcinoembryonic antigen) were not considered measurable. On study evaluation consisted of physical examination, baseline laboratory tests, chest x-ray, bone scan, and measurement of marker areas. Patients were required to be ambulatory, with a Karnofsky status of > 50, leukocyte count ⩾ 4000/mm^3, and platelet count ⩾ 100,000/mm^3. Patients could not have received antineoplastic therapy (chemotherapy, surgery, radiation therapy, or immunotherapy) in the preceding 4 weeks or previous therapy with any IFN preparation or VBL.

Drug Administration

Patients received 3×10^6 IU/day of αIFN im 5 days/week (Monday through Friday). VBL was administered weekly on Day 1 of each 5-day course of αIFN. The starting dose was 0.15 mg/kg iv for the first five patients. Because of hematologic toxicity (discussed below), all subsequent patients received a starting dose of 0.1 mg/kg iv. Doses were modified for hematologic toxicity as described below. This IFN schedule was chosen to facilitate outpatient administration and to allow a weekly recovery period during which the toxicity of IFN might abate. The dosage of IFN chosen is similar to that which has previously shown activity in studies of osteogenic sarcoma, non-Hodgkin's lymphoma, breast cancer, multiple myeloma, and renal cell carcinoma (1,6,9). The dosage of VBL was chosen because reports indicate that responses are noted when given weekly at a dose of 0.1–0.3 mg/kg, with higher doses usually associated with higher response rates (8).

Patients who had a complete (CR) or partial (PR) response to αIFN/VBL therapy were treated until their disease progressed or until severe toxicity limited the safe administration of the therapy. Patients with minimal response or stable disease after 20 weeks of therapy were removed from the study. Patients who had progressive disease or severe toxicity were removed from the study and observed. Patients were evaluated for toxicity prior to each course of αIFN/VBL by weekly blood cell counts and every 3 weeks by screening chemistry panels. Bone marrow examinations were not performed. Patients were examined every 3 weeks or as indicated. Marker lesions measured on chest roentgenographs and by physical examinations were evaluated every 3 weeks. Marker lesions measured by computed tomography or scan were evaluated every 6 weeks.

Dosage Modification

αIFN/VBL therapy was modified for toxicity as follows: if severe hematologic toxicity occurred (wbc count ⩽ 1500/mm^3, polymorphonuclear neutrophil count ⩽ 500/mm^3, or platelet count ⩽ 50,000/mm^3), therapy was discontinued and restarted at a 100% IFN dose and a 0.1-mg/kg VBL dose given every other week after hematologic recovery (wbc count ⩾ 3000/mm^3, polymorphonuclear neutrophil count ⩾ 1000/mm^3, and platelet count ⩾ 85,000/mm^3). If severe hematologic toxicity occurred at the above reduced dose level, therapy resumed upon hematologic recovery with a 50% IFN dose and a 0.1-mg/kg VBL dose given every other week. If severe hematologic toxicity occurred at this level, the patient was removed from the study. VBL therapy was discontinued upon the development of neurotoxicity characterized by any of the following: absent deep tendon reflexes, moderate to severe paresthesias, severe neuritic pain, or difficulty with ambulation. αIFN/VBL was discontinued with the development of severe malaise requiring hospitalization.

Response Criteria

Response to therapy was classified as follows. CR was defined as the disappearance of all evidence of disease lasting ⩾ 4 weeks. PR was defined as a ⩾ 50% decrease in the sum of the products of diameters of marker lesions (or for malignant hepatomegaly, the sum of the extents below the xyphoid and the costal margins in the midclavicular lines) lasting ⩾ 4 weeks, without the appearance of new lesions or the growth of old lesions. Minimal response was defined as clear objective shrinkage of disease of insufficient magnitude to be classed as PR but lasting ⩾ 4 weeks, without the appearance of new lesions or the growth of old lesions. Disease progression was defined as the appearance of new lesions, tumor-induced death, or an increase of ⩾ 25% in measured tumor areas. All other changes in measurement were considered as stable disease.

Patients who received at least one dose were considered evaluable for toxicity. Patients receiving 2 weeks of therapy were considered evaluable for efficacy.

RESULTS

Patient Entry

Twenty-four patients were entered in this study. Patient characteristics are shown in table 1. All patients were considered evaluable for toxicity. One patient had received < 2 weeks of therapy when he experienced a

TABLE 1.—Patient characteristics and response

	No. of patients (%)
Total	24
Evaluable for toxicity	24
Evaluable for response	23
Sex	
Male	16
Female	8
Median age in yrs (range): 61 (42-76)	
Median performance status (range): 90 (60-100)	
Responders*: 96 (90-100)	
Nonresponders†: 89 (60-100)	
Previous chemotherapy	
None	23 (100)
Sites of metastatic disease	
Lung	22 (92)
Lung only	11 (46)
Bone	9 (38)
Kidney, renal fossa, and retroperitoneum	6 (25)
Subcutaneous	5 (21)
Liver	2 (8)
Response	
CR	0 (0)
PR	3 (13)
Minimal	5 (22)
Stable disease	4 (17)
Disease progression	11 (48)

* CR, PR, and minimal response.
†Stable disease and disease progression.

pathologic fracture of his hip requiring surgical intervention and radiation therapy. αIFN/VBL was discontinued at this point and was not subsequently restarted. This patient was considered not evaluable for efficacy. The 23 patients evaluable for efficacy received a median of 64 doses of IFN (range, ten to 133 doses) and 11 doses of VBL (range, three to 25 doses).

Efficacy

Of 23 patients evaluable for response, we observed an objective response rate of 13%. This consisted of no CRs and three PRs lasting 1.5, 8+, and 12+ months. Five additional patients (22%) obtained minimal responses lasting 4, 8+, 6, 9, and 13+ months. Seven of eight responding patients had measurable disease in the lung as the predominant site of metastatic disease. One of the patients obtaining a PR had surgical resection of residual abdominal disease following maximal response to therapy. Only one partial responder remains on active treatment. Three responders (one with PR; two with minimal response) received the starting VBL dose of 0.15 mg/kg/week. The remaining responders (two with PR; three with minimal response) were started at the lower 0.1-mg/kg/week dose. Four patients (17%) had stable disease. Two of the patients with stable disease had therapy discontinued at 3 and 4 weeks, respectively, secondary to severe toxicity (*see* below). The remaining two patients had 20 weeks of therapy and then had treatment discontinued. Eleven patients had disease progression while receiving therapy, with a median time to progression of 9.3 weeks (range, 3-14.5).

Toxicity

Table 2 shows the frequency and severity of side effects. Fever (≥ 38.5°C) was common, occurring in 96% of patients; it was controllable with acetominophen alone. Malaise was also common, occurring in 96% of patients. Unlike our previous IFN-alone trial, 8% of patients in this study required hospitalization for intolerable malaise and discontinuation of treatment. These patients had no concomitant neurotoxicity. Both patients recovered to their pretreatment performance status and left the hospital with stable disease. Nausea/vomiting occurred in nearly three-fourths of patients and was mild. Four patients had hair loss, one of these being total alopecia. Mild to moderate hepatic toxicity, manifest as elevations of SGOT and SGPT, occurred in 42% of patients

TABLE 2.—Toxicity

Toxicity	No. of patients (%)
Hematologic	
Nadir wbc count (cells/mm^3)	
≥ 4000	6 (25)
3000-3999	2 (8)
1500-2999	10 (42)
1000-1499	6 (25)
Nadir granulocyte count (cells/mm^3)	
≤ 1500	7 (29)
1000-1499	3 (12)
750-999	4 (17)
500-749	4 (17)
≤ 500	6 (25)
Nadir platelet count (cells/mm^3)	
> 150,000	21 (88)
100,000-150,000	3 (12)
Hepatic (SGOT)	
None	13 (54)
Mild-moderate	10 (42)
Severe (2-4 × baseline)	1 (4)
Nausea/vomiting	
Alopecia	
Mild	3 (12.5)
Complete	1 (4)
Fever	23 (96)
Malaise	23 (96)
Intolerable (requiring hospitalization)	2 (8)
Neurotoxicity	5 (21)
Severe	1 (4)
Intolerable	2 (8)

and was not associated with jaundice or other clinical evidence of hepatic injury. One patient developed asymptomatic transaminase elevations four times normal, which reverted to normal when therapy was discontinued. Neurotoxicity occurred in five patients (21%). Two patients experienced mild paresthesias but continued receiving therapy without progression of symptoms. Three patients experienced toxicity requiring discontinuation of VBL, two patients developed loss of deep tendon reflexes and moderate paresthesias, and one patient developed foot drop and disabling neuritic pain after 3 weeks of therapy. Neurotoxicity had no apparent relationship to the level of hepatic dysfunction. Hematologic toxicity was frequent, with 59% of patients developing granulocyte counts $< 1000/mm^3$ and a full 25% developing granulocyte counts $< 500/mm^3$. Two of five patients had granulocytopenia < 500 cells/mm^3 at a starting dose of 0.15 mg/kg/week of VBL. Eight patients (33%) required a lowering of VBL dose scheduling to 0.1 mg/kg/week because of severe hematologic toxicity. Time to hematologic recovery in patients requiring dose attenuation averaged 1 week and occurred independent of the degree of granulocytopenia. Despite this severe toxicity, no patient required antibiotic support or hospitalization. Mild thrombocytopenia occurred in 12% of patients and was not associated with a bleeding diathesis. Hematologic toxicity occurred without apparent correlation to the level of hepatic dysfunction. No fatal toxicity occurred.

DISCUSSION

The αIFNs are biological response modifiers that have demonstrated some activity against a variety of malignancies including breast cancer, multiple myeloma, non-Hodgkin's lymphoma (9), renal cell carcinoma (1-6), hairy cell leukemia (10), and Kaposi's sarcoma in patients with the acquired immunodeficiency syndrome (11). The mechanisms of antineoplastic effects are not clear but may include direct cytotoxicity or indirect cytotoxicity mediated by lymphocyte or macrophage functions.

Patients with renal cell carcinoma currently have few therapeutic options once the disease has become metastatic (12). In practice, progestational agents are the mainstay of therapy and give rise to a small proportion of objective responses of usually short duration. Single-agent chemotherapy and combination chemotherapy may give slightly higher response rates but as yet show no clearly defined improvement in patient survival (13). IFNs have induced objective responses (CR + PR) in 7%-28% of patients with renal cell carcinoma. Patients with pulmonary disease only, with prior removal of their primary tumor and with good performance status, may be more likely to respond than patients with more widely metastatic disease. As yet, no group has reported improvement in either overall survival or survival of responders as compared to nonresponders. The UCLA experience with αIFN was recently reported and demonstrated a 16.5% objective response rate (6). A current update of that study through June 1984 with a minimum follow-up time of 21 months demonstrates a median survival of 16 months for responders and a median survival of 10 months for nonresponders. With the existent sample size this is not of statistical significance ($P = 0.21$ by generalized Wilcoxon analysis).

Recent evidence using the human tumor stem cell assay has suggested significant synergistic activity against in vitro colony formation with the combination of IFN and VBL (7). This contrasts with older data suggesting that VBL can inhibit IFN-induced antiviral activity, possibly by the inhibition of an IFN receptor (14). Additionally, VBL as a single agent has long been felt to have small but definite activity against renal cell carcinoma. In a collected series of 135 patients, a response rate of 25% was noted. In 39 patients who received 0.2-0.3 mg/kg/week, the response rate was 31%. In 96 patients who received < 0.2 mg/kg/week, only 15% responded, suggesting a possible dose response effect (8). Based on the above information we combined IFN and VBL as treatment for patients with advanced renal cell carcinoma. Our initial schedule of 3×10^6 IU/day of αIFN im daily 5 days out of 7 with 0.15 mg/kg/week of VBL required a significant dose modification because of toxicity. Ultimately, a schedule of similar IFN dosage and 0.1 mg/kg of VBL either weekly or biweekly was better tolerated. The combination regimen yielded an overall objective response rate of 13% (95% confidence level; response rate $\leq$ 27%), with an additional 22% minimal response rate. This is very similar to those results achieved here and elsewhere with IFN alone. In comparing the toxicity in the current trial with that obtained in a previous study of the same type, dose, and schedule of IFN alone, marked differences were observed. Severe granulocytopenia occurred in 42% of patients currently, compared to 5% in our previous study. No patient in the IFN-alone study experienced granulocyte counts $< 500/mm^3$, whereas 25% did so currently. Similarly, neurotoxicity was absent from our IFN-alone study, while 12% of patients receiving IFN plus VBL experienced significant neurotoxicity. Fatigue/malaise was comparable in incidence in both trials but only in the current study did it result in hospitalization and discontinuation of therapy in 8% of patients. These differences indicate that this current regimen is more toxic than IFN alone. Hematologic and neurotoxicity in this study exceeded expected toxicity for VBL alone in the dosages administered (15). The mechanism for this increased toxicity is unknown. While the increased hematologic toxicity and malaise due to the two drugs may be either additive or synergistic, the increased neurotoxicity suggests synergism or potentiation of VBL effects. VBL is metabolized in the liver; thus, IFN-induced liver dysfunction could potentially increase

VBL toxicity on both the hematopoietic and peripheral nervous systems. No correlation with chemical evidence of hepatic toxicity could be demonstrated in the present study. The demonstrated in vitro antineoplastic synergism of IFN and VBL may reflect similar synergy of effects on the hematopoietic and peripheral nervous systems. Not all toxicity was clearly increased, however. Hepatic dysfunction, fever, nausea, and thrombocytopenia were similar in frequency and severity to our IFN-alone study.

In conclusion, in this phase I–II study of αIFN plus VBL as treatment of advanced renal cell carcinoma, we observed comparable response rates but increased toxicity as compared to our previous experience with IFN alone. While αIFN administered daily 5 days out of 7 with VBL given biweekly may offer a better tolerated schedule for future trials, we do not find evidence that improvement in response rates will result.

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Bioavailability, Pharmacokinetics, and Clinical Effects of an Oral Preparation of Etoposide[1,2]

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Bioavailability of an oral preparation of the antineoplastic drug etoposide (VP-16) was studied in 13 patients with advanced malignancies. An initial pilot study involving three patients suggested that approximately 50% of an orally administered dose was absorbed. Ten additional patients were randomized to receive either 100 mg/m^2/day iv or 200 mg/m^2/day orally. Three weeks later, the alternate dose schedule was administered. Plasma samples were assayed for VP-16 using a high-pressure liquid chromatography technique. Comparison of the area under concentration-time curves (C × t) revealed that 17%–72% (mean, 52%) of an orally administered dose was absorbed. Absorption was < 40% for only one patient. For oral and iv preparations, mean peak plasma VP-16 concentrations were 9.6 and 13.0 μg/ml, mean α-half-lives were 0.96 and 0.82 hour, mean β-half-lives were 7.2 and 6.8 hours, mean C × t values were 75.9 and 75.3 mg/L/hour, mean plasma clearances were 1.44 and 1.45 L/hour/m^2, and mean extrapolated volumes of distribution were 15.2 and 16.9 L/m^2, respectively. The half-life for oral absorption was 0.44 hour and peak plasma concentrations were noted 0.5–3 hours after oral drug administration. Granulocyte count nadirs tended to be lower in patients with high C × t values and low plasma clearance values. Granulocytopenia was dose-limiting. Gastrointestinal toxicity was extremely mild. We recommend doses of oral VP-16 of 800 mg/m^2/course over 3–5 days for patients with a moderate amount of prior treatment. It is probable that previously untreated patients will tolerate a higher dose and that heavily pretreated patients will require a lower dose. [Cancer Treat Rep 69:269–273, 1985]

The antineoplastic agent etoposide (VP-16) has marked activity against small cell undifferentiated carcinoma of the lung (1,2) and is also active against several other malignancies, including diffuse histiocytic lymphoma, acute leukemia, carcinoma of the breast, and carcinoma of the testicle (1,2).

The major method of administering VP-16 has been by iv infusion. However, various oral preparations have also been utilized (1,3–5). A liquid preparation in the form of drinking ampules provides a bioavailability of up to 90% (1,3–5), but patients are very reluctant to use it because of its unpleasant taste. Lipophilic capsules also produced severe gastrointestinal toxicity and absorption was very erratic (1,5). More recently, a hydrophilic gelatin capsule preparation has been found to produce an acceptable degree of gastrointestinal toxicity and an average of around 50% of an administered dose is absorbed (1,3,4).

The antitumor efficacy of VP-16 is highly schedule-dependent in animal tumor systems, with multiple-day administration being superior to single-day administration (2). Unfortunately, it is frequently difficult to have patients return for iv treatments on multiple successive days, and oral preparations can greatly facilitate administration of drugs on a multiple-day schedule. Because of this, an oral preparation of VP-16 was formulated and bioavailability and clinical trials were initiated.

MATERIALS AND METHODS

Soft gelatin VP-16 capsules in 10- and 50-mg sizes

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were manufactured at R. P. Scherer Inc, Windsor, Ontario, Canada. The capsules and parenteral VP-16 were supplied by Bristol Laboratories of Canada.

Thirteen patients were studied, all of whom gave informed consent. Diagnoses included small cell undifferentiated carcinoma of the lung (two patients), squamous cell carcinoma of the lung (three), adenocarcinoma of the lung (two), and large cell undifferentiated carcinoma of the lung, nasopharyngeal carcinoma, and adenocarcinomas of the pancreas, colon, kidney, and unknown primary (one patient each). Ten of the patients had previously received chemotherapy and eight had previously been irradiated. Only one patient had had no prior treatment. Performance status by Eastern Cooperative Oncology Group criteria was 1 for eight patients, 2 for four, and 3 for one. One patient had abnormal renal function, with serum creatinine of 2.2 mg/100 ml and BUN of 36 mg/100 ml. One patient had an elevated serum bilirubin (1.9 mg/100 ml) during his second course of treatment, although it had been normal during his first course.

To determine an appropriate dose of oral medication for further study, the first three patients were treated initially with iv 1-hour infusions of VP-16 at a dose of 100 mg/m^2/day for 4 days. For the second course of treatment 3 weeks later, the three patients received, respectively, VP-16 at doses of 150, 175, and 200 mg/m^2 orally on an empty stomach daily for 4 days. Based on the information from this pilot study, ten additional patients were randomized to receive VP-16 either orally (200 mg/m^2/day) or by iv 1-hour infusion (100 mg/m^2/day) during their first course of treatment and were given the medication by the alternate route during their second course of treatment. Blood samples were obtained prior to treatment and following administration of the capsules or initiation of the iv infusion at 15, 30, 45, 60, 75, and 90 minutes and at 2, 4, 6, 8, 24, and 25 hours. For technical reasons, blood samples were obtained at 3, 5, 7, and 23 hours instead of at 4, 6, 8, and 25 hours in a few patients. Assays were then performed using a modification of the method of Evans et al (6).

Plasma samples (1.5-ml) were combined with an equal volume of cold saturated ammonium sulfate. Ethyl acetate (5 ml) was then mixed with the above mixture by vortexing for 30 seconds. Following centrifugation at 3000 rpm for 15 minutes, the ethyl acetate layer was removed. Methylene chloride (5 ml) was then added to the sample and vortexed for 30 seconds. Following centrifugation at 5000 rpm for 10 minutes, the methylene chloride layer was removed and added to the ethyl acetate layer in a 15-ml pyrex centrifuge tube. Evaporation was conducted at 35°C under a vacuum of 23 cm Hg using a Buchler Vortex Evaporator. The dried extract in the tube was washed first with 2 ml of methylene chloride and again evaporated to dryness. This was repeated twice more using first 1 ml and then 0.5 ml of methylene chloride. Two-hundred microliters of 100% acetonitrile was added to the final extract and vortexed for 30 seconds. Ten microliters of the sample was loaded onto a Water's high-pressure liquid chromatography system equipped with a U6K injector and a μBondapak phenyl column. The mobile phase consisted of 25% acetonitrile, 74% water, and 1% glacial acetic acid running at 2.0 ml/minute. Detection was carried out using both an ultraviolet detector at 254 nm as well as a Bioanalytical Systems electrochemical detector at 0.75 volts (oxidative mode).

Pharmacokinetic parameters were calculated using curve peeling techniques and bioavailability of the oral preparation was determined by comparing the area under the concentration-time curve (C × t) for oral versus iv administration in individual patients.

Patients had weekly determinations of wbc, granulocyte, and platelet counts and had serum biochemistries done every 3 weeks while in the study. Patient interviews and physical examinations were performed every 3 weeks and appropriate radiological studies to evaluate response were repeated every 6–9 weeks. Partial remission was defined as a $\geq$ 50% decrease in tumor size; disease progression was defined as a $\geq$ 25% increase in tumor size; and stable disease was defined as improvement < 50% and disease progression < 25%.

RESULTS

Pharmacokinetics and Bioavailability

The initial three patients were treated first with iv VP-16 at a dose of 100 mg/m^2/day followed in 3 weeks by oral VP-16 at doses of 150, 175, and 200 mg/m^2, respectively; the ratios of the C × t for oral versus iv VP-16 were 0.7, 1.00, and 0.88, respectively. Thus, it was felt appropriate to continue the study using oral VP-16 doses of 200 mg/m^2/day. Pharmacokinetic parameters are shown in table 1. Means were determined using the ten evaluable patients receiving an oral VP-16 dose of 200 mg/m^2. There were no major differences between oral and iv administration in half-lives, clearance, or volume of distribution. Peak plasma concentrations of drug were generally lower with oral than with iv administration. Peak plasma concentrations occurred between 0.5 and 3 hours following oral dosing. In most patients, the peak plasma concentration occurred at around 1 hour after treatment. For four of the ten patients given oral VP-16, plasma concentrations declined biexponentially after peaking, while in six the decline was monoexponential. All patients given iv VP-16 had biexponential decreases in plasma concentrations.

The pharmacokinetic parameters for the two patients who received lower doses of oral VP-16 during the

initial pilot study were similar to the parameters for the ten patients whose values were included in the calculations of means.

Administration of 200 mg/m^2 of VP-16 orally resulted in drug exposure comparable to 100 mg/m^2 administered iv. For individual patients, the ratio of the oral versus iv C × t ranged from 0.35 to 1.44, with the mean ± SD being 1.05 ± 0.34. Hence, the fraction of orally administered drug that was absorbed was 0.52 ± 0.17 (range, 0.17-0.72). The fraction absorbed was < 0.4 in only one patient.

Values for the patient who had an elevated serum bilirubin were excluded from calculations of mean pharmacokinetic parameters. During his first course of treatment, which was administered iv, his serum bilirubin was within normal limits and his pharmacokinetic parameters were similar to those seen with other patients. During his second course of treatment, which was administered orally, his serum bilirubin was 1.9 mg/100 ml. Following this course, plasma concentrations peaked after 6 hours at 2.1 μg/ml and remained essentially stable at that level over the entire 24-hour sampling period. Pharmacokinetic parameters from the single patient with abnormal renal function (serum creatinine, 2.2 mg/100 ml) were similar to the parameters for other patients.

Pharmacokinetics were the same whether patients received the drug orally or iv first. No metabolites were detected in the serum of any patient.

TABLE 1.—Pharmacokinetic parameters for VP-16 following oral or iv administration

Patient No.	Route	Dose (mg/m^2)	$t_{1/2\alpha}$ (hrs)	$t_{1/2\beta}$ (hrs)	C × t (mg/L/hr)	Clearance (L/hr/m^2)	Volume of distribution (L/m^2)	Peak plasma concentration (μg/ml)
1	Oral	150	NE*	7.7	71.5	1.41	15.6	7.0
	Iv	100	0.67	5.8	71.4	1.40	11.7	10.2
2	Oral	175	0.76	5.8	60.1	1.17	9.7	8.5
	Iv	100	0.39	5.8	85.1	1.18	9.8	14.5
3†	Oral	200	NE	NE	NE	NE	NE	2.1
	Iv	100	1.24	9.9	140.7	0.71	10.2	15.8
4	Oral	200	0.88	6.9	72.7	1.21	12.1	10.4
	Iv	100	0.57	5.8	82.9	1.21	10.1	14.7
5	Oral	200	NE	5.0	28.9	1.18	8.4	3.5
	Iv	100	1.26	8.7	82.7	1.21	15.1	11.0
6	Oral	200	NE	6.3	49.2	2.77	25.2	5.5
	Iv	100	0.85	6.3	35.9	2.79	25.3	12.7
7	Oral	200	1.28	7.7	86.8	1.48	16.4	15.8
	Iv	100	0.28	5.3	67.3	1.49	11.4	18.8
8	Oral	200	0.76	5.8	66.7	1.44	12.0	9.7
	Iv	100	0.31	4.3	69.3	1.44	9.0	13.0
9	Oral	200	0.92	5.8	87.3	0.96	8.0	15.9
	Iv	100	0.26	6.3	103.7	0.97	8.8	16.1
10	Oral	200	NE	6.9	114.4	0.89	8.9	11.2
	Iv	100	0.52	6.3	111.7	0.90	8.1	15.4
11	Oral	200	NE	7.7	65.4	1.47	16.3	9.0
	Iv	100	1.26	6.9	67.7	1.48	14.8	8.6
12	Oral	200	NE	11.6	91.0	1.54	25.6	8.1
	Iv	100	1.05	7.7	65.1	1.54	17.1	7.1
13	Oral	200	NE	8.7	96.4	1.54	18.7	7.2
	Iv	100	1.87	9.9	67.1	1.49	21.3	7.7
Mean ± SD	Oral		0.96 ± 0.22	7.2 ± 1.9	75.9 ± 24.8	1.44 ± 0.52	15.2 ± 6.3	9.6 ± 4.0
	Iv		0.82 ± 0.53	6.8 ± 1.6	75.3 ± 21.4	1.45 ± 0.52	16.9 ± 9.9	13.0 ± 5.0

*NE = not evaluable. No $t_{1/2\alpha}$ could be determined for many patients after oral drug administration, since the drug concentration declined monoexponentially.

†Values for this patient were not included in determination of means. He had normal serum bilirubin during his first (iv) treatment, but elevated bilirubin during his second (oral) treatment. Following his oral drug dose, plasma concentrations peaked at 6 hrs, but then failed to decline over the 24-hr sampling period.

Toxicity

Hematological toxicity is shown in table 2. We had previously found that patients who had had no prior treatment would tolerate an iv VP-16 dose of 100 mg/m^2 for 5 days (J. Maroun, unpublished data). Since most of the patients in this study had previously been treated, we initially gave our patients only 4 days of VP-16 (100 mg/m^2/day iv or 200 mg/m^2/day orally). Since granulocyte count nadirs of $< 1000/\mu l$ were seen in six of 13 courses on a 4-day schedule, subsequent patients received these daily doses for only 3 days. Two of six courses given over 3 days were associated with granulocyte count nadirs of $< 1000/\mu l$. In both cases, the patients had been very heavily pretreated. Oral and iv courses were similar with respect to the degree of myelosuppression induced. Three courses of chemotherapy were associated with fever during neutropenia. In each case, full recovery occurred with antibiotics. There were no episodes of bleeding during thrombocytopenia.

Granulocyte count nadirs were generally lower for patients with large C × t values and low clearances, although the association was quite weak (correlation coefficient was 0.2 for C × t and 0.4 for clearance). If patients are divided into two groups so that one-half of the patients are above and one-half of the patients are below the median for C × t, granulocyte count nadirs for the group with the higher C × t values are lower than those for the group with the lower C × t values ($P < 0.05$ by Student's t test). The same is true when patients are divided into two equal groups according to clearance, except that lower granulocyte count nadirs were associated with lower drug clearances. Patients with longer VP-16 elimination half-lives also tended to have lower granulocyte count nadirs, but the association was not statistically significant. Nausea was nonexistent in 62% of iv courses and 80% of oral courses, mild in 23% of iv courses and 10% of oral courses, and moderate in 15% of iv courses and 10% of oral courses. Alopecia developed in most patients. No other toxic effects were noted.

Response

One patient with small cell undifferentiated carcinoma of the lung and one with large cell undifferentiated carcinoma of the lung achieved partial remission. One patient with squamous cell carcinoma of the lung had a mixed response. Four patients had stable disease for > 12 weeks, including two with adenocarcinoma of the lung and one each with hypernephroma and nasopharyngeal carcinoma. The patient with the nasopharyngeal carcinoma had moderate symptomatic improvement. The remainder of the 13 patients experienced tumor progression within 6 weeks of initiating treatment, although one patient with squamous cell carcinoma of the lung subsequently improved when cisplatin was added to the VP-16.

DISCUSSION

The pharmacokinetic parameters of VP-16 noted in this study were similar to those noted by others (3,4,7-10). For other high-pressure liquid chromatography studies, values have ranged from 0.79 to 1.4 hours for α-half-lives, from 3.0 to 12.3 hours for β-half-lives, from 0.48 to 1.62 L/hour/m^2 for plasma clearance, and from 7 to 50 L/m^2 for volume of distribution. Studies using radiochemical techniques have reported somewhat different results (11).

Our results for bioavailability following administration of oral VP-16 were similar to recently reported results for other VP-16 capsule preparations (1,3,4). Like others, we found that approximately 50% of an orally administered dose is absorbed, although occasionally < 40% or > 70% will be absorbed. Hence, we recommend that the oral dose of VP-16 should be twice as high as an iv dose would be in the same situation. However, one must be prepared to escalate the dose with subsequent courses if no dose-limiting toxicity is noted. Likewise, patients at exceptionally high risk of developing serious toxicity should be treated cautiously, since particularly severe myelosuppression could develop if they absorb more of the drug than anticipated. If oral VP-16 is being used as a single agent, we recommend initially using 800 mg/m^2 divided over 3-5 days for most patients. Heavily pretreated patients probably should receive a lower dose. It is probable that patients with no prior treatment would tolerate a somewhat higher dose. However, caution must be exercised in view of the occasional unpredictably high absorption of

TABLE 2.—Hematological toxicity following administration of oral (200 mg/m^2/day) and iv (100 mg/m^2/day) VP-16

Route	No. of days/ course	Granulocyte count nadir (× 10^3 cells/μl)		Platelet count nadir (× 10^3 cells/μl)	
		Median	Range	Median	Range
Oral	3	0.6	0.01-2.9	149	53-435
	4	1.0	0.01-4.6	175	75-556
Iv	3	2.4	1.4-4.4	320	190-513
	4	0.6	0.01-1.9	150	93-236

the drug. Moreover, we have not determined whether or not food in the stomach affects absorption, since all of our patients took the oral preparation on an empty stomach. In addition, each of our patients was studied only during a single course of treatment. It is always possible that oral absorption could change with repeated treatment. It is also unknown whether the bioavailability would be the same for different oral doses of the tested capsules. However, our two patients treated with oral doses of 150 and 175 mg/m^2 had bioavailability results quite comparable to those noted in patients receiving oral doses of 200 mg/m^2.

Patient acceptance of this form of treatment was exceptionally good. Few patients vomited following oral VP-16 and most had no nausea whatsoever. Hence, gastrointestinal toxicity with oral VP-16 is similar to that with iv VP-16.

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Single-Dose Pharmacokinetic Study of 13-*cis*-Retinoic Acid in Man[1,2]

Jean-Guy Besner,[3,*] Robert Leclaire,[3] Pierre Band,[3,4] Sylvain Meloche,[3] Michèle Deschamps,[5] Sylvie Mailhot,[3] Richard Moisan,[3] and Genevieve Diorio[5]

A pharmacokinetic study of 13-*cis*-retinoic acid was performed in nine patients following administration of a single oral dose of 80 mg. An average lag time of 1.2 hours was observed, followed by fast absorption, with a mean half-life of 0.5 hour. Peak plasmatic concentration of 733 ng/ml occurred at 2.3 hours. The disposition profile showed a rapid distribution half-life of 1.3 hours and a terminal elimination half-life of 24.7 hours. No 13-*cis*-retinoic acid was detected unchanged in urine. An important interpatient variability was noted. [Cancer Treat Rep 69:275–277, 1985]

Retinoids are essential for the normal growth and differentiation of epithelial tissues (1-3). These compounds exhibit prophylactic and therapeutic activity against epithelial neoplasms in animals (4,5). In humans, retinoids have been shown to induce regressions of urinary bladder papillomas, oral leucoplasias, and fibrocystic masses of the breast (5,6). In addition, retinoids have clinical indications for a variety of cutaneous disorders (7). 13-*cis*-Retinoic acid (13-*cis*-RA), a synthetic analog of vitamin A, is currently used in the treatment of cystic acne (8). Based on these observations, we performed a phase I trial of 13-*cis*-RA in cancer patients (9). This paper reports the pharmacokinetics of 13-*cis*-RA after single oral-dose administration.

MATERIALS AND METHODS

Patients and drug administration.—Nine male patients with stage I or II head and neck malignancies were hospitalized on Day 1 of a phase I trial of 13-*cis*-RA to undergo a pharmacokinetic study. All patients had fully recovered from the effects of surgery and/or radiation therapy; had normal hemogram, creatinine clearance, and liver function tests; and were not taking any vitamin A medication. Patients were kept fasting from midnight until 4 hours after drug administration. A single dose of 80 mg (two capsules of 40 mg) of 13-*cis*-RA was given orally with 200 ml of water.

Sample collection.—Blood samples were drawn in heparinized vacutainers at 0 hour, every 15 minutes for 2 hours, every 30 minutes for the next 2 hours, hourly for 6 hours, and at 12, 14, 16, 24, and 48 hours after drug intake. The tubes were immediately centrifuged and plasma was kept frozen in amber tubes at -30°C until analysis. Urine samples were collected at 0, 0-4, 4-8, 8-12, 12-24, and 24-48 hours and were stored at -30°C until analysis. All biological samples were collected under subdued red lights.

Analytical assay.—Plasma and urine concentrations of 13-*cis*-RA were determined using a specific high-performance liquid chromatographic assay (10). The samples were extracted twice with a mixture of hexane-methylene chloride-isopropanol (80:19:1) and analyzed by reverse-phase high-performance liquid chromatography. The mobile phase consisted of acetonitrile-1% ammonium acetate in water (80:20). This method allowed a complete separation of 4-oxo-13-*cis*-RA, 13-*cis*-RA, *trans*-RA, and retinol with retention times of 1.9, 2.7, 3.5, and 4.0 minutes, respectively. Recovery was 85% and the variability was < 10%. The plasma concentrations of 4-oxo-13-*cis*-RA were not measured in this study.

Pharmacokinetic analysis.—The choice of model and preliminary parameter estimates were obtained with the aid of the program AUTOAN (11). Final determination of all pharmacokinetic parameters was done by fitting the plasma level data to the exponential equations

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[2]13-*cis*-Retinoic acid was supplied by Hoffmann-La Roche Inc, Nutley, NJ.

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for the chosen model using the nonlinear regression program NONLIN (11).

RESULTS

For all patients, the plasma level data were best described by a linear two-compartment open model with first-order absorption. The individual values of the 13-*cis*-RA kinetic parameters in patients are shown in table 1. In all cases, a lag time ranging from 0.84 to 1.71 hours was observed. Absorption was rapid as indicated by a mean absorption half-life ($t_{1/2}Ka$) of 0.48 hour. The peak plasma concentration (C_{max}) varied from 453 to 1149 ng/ml, with a mean of 733 ng/ml; the mean time to reach that concentration (T_{max}) was 2.31 hours. A rapid distribution half-life of 1.32 hours was observed, with an average ratio of 3.49 between the rate constant for the transfer of 13-*cis*-RA from the central to the peripheral compartment (K_{12}) and the rate constant for the reverse process (K_{21}). The mean terminal plasma half-life was 24.68 ± 12.00 hours, indicating a slow elimination of the drug. The area under the plasma level curve (AUC) varied from 2568 to 13,725 ng/ml·hr. Plasma levels of *trans*-RA were also measured and were found to be < 5% of the concentrations of 13-*cis*-RA. Urine samples were assayed for unchanged 13-*cis*-RA content. No 13-*cis*-RA was detected in any of the collected samples.

DISCUSSION

The results of this study show that, after oral administration in man, 13-*cis*-RA is rapidly absorbed by a first-order process with a lag time prior to the appearance of the drug in the systemic circulation. The disposition profile is biexponential, with rapid distribution and slow elimination.

The lag time may be explained by biopharmaceutical considerations as the time needed for the liberation and dissolution of the drug in gastrointestinal fluids. Frequent sampling times are required to clearly define this lag time and the fast absorption process. Since no data are available from iv administration in man, the fraction of the dose that actually reaches the circulation and the volume of distribution could not be determined in our study. The mean ratio of 3.49 between the distribution rate constants K_{12}/K_{21} suggests that 13-*cis*-RA is widely distributed in peripheral tissues. This high tissular affinity has been shown by Wang et al (12) and probably reflects the high liposolubility of the drug. The mean elimination half-life of 24.7 hours is in agreement with the findings of Goodman et al (13). However, interpatient variations are important, with individual values ranging from 7.7 to 48.5 hours. Since the value of Ka was approximately 50 times larger than β, it may be assumed that absorption has no significant influence on the evaluation of the elimination constant. No unchanged 13-*cis*-RA was detected in any of the patients' urine samples. The elimination constant would thus reflect extensive hepatic elimination. The major metabolite of 13-*cis*-RA in human blood was identified as 4-oxo-13-*cis*-RA (14).

The AUC values were found to be extremely variable, which suggests that the extent of systemic absorption of 13-*cis*-RA from the capsules is variable. Enterohepatic circulation could have also contributed to the observed variations in plasma concentrations and in elimination half-life (13). The pharmacokinetic parameters estimated in this study are in good general agreement with those published by Colburn et al (15). In opposition to the results of Goodman et al (13), who reported a 10%–30% in vivo isomerization of 13-*cis*-RA to *trans*-RA, our results indicate that this metabolic transformation is very minor and might be an artifact due to the extraction procedure.

The present study shows important interpatient variability in plasma concentrations of 13-*cis*-RA after a single oral dose. In view of these findings, it might be reasonable to expect similar variations regarding long-term dosing. However, further studies are necessary to evaluate the pharmacokinetics of 13-*cis*-RA after long-term administration.

TABLE 1.—Pharmacokinetic parameters of 13-*cis*-RA after oral administration in patients

Patient No.	Dose		Lag time (hrs)	$t_{1/2}Ka$ (hrs)	C_{max} (ng/ml)	T_{max} (hrs)	AUC (ng/ml·hr)	$t_{1/2\alpha}$ (hrs)	K_{12}/K_{21}	$t_{1/2\beta}$ (hrs)
	mg	mg/m²								
1	80	50.00	0.87	0.98	801.1	3.00	9345.5	1.94	5.26	48.50
2	80	54.05	1.21	0.28	519.4	2.50	6580.5	2.82	2.08	35.33
3	80	42.10	1.48	0.04	701.7	1.75	5960.3	0.60	3.12	21.57
4	80	41.05	1.71	0.10	693.4	2.00	6857.0	0.90	2.31	19.33
5	80	42.10	0.96	0.39	453.1	1.75	3526.6	0.84	3.05	14.87
6	80	47.10	0.84	0.45	707.2	2.00	6724.9	0.75	6.70	24.45
7	80	51.61	1.20	0.98	1107.7	3.50	13,725.5	1.83	2.15	20.35
8	80	41.24	0.95	0.45	464.1	1.75	2568.6	0.87	1.70	7.67
9	80	48.50	1.21	0.66	1149.2	2.50	5737.4	1.37	5.00	30.03
Mean		46.42	1.16	0.48	733.0	2.31	6780.7	1.32	3.49	24.68
SD		4.95	0.29	0.34	254.4	0.62	3258.6	0.74	1.75	12.00

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Sequence-Dependent Synergism Between Dichloromethotrexate and Cisplatin in a Human Colon Carcinoma Cell Line[1,2]

Alberto F. Sobrero and Joseph R. Bertino[3,*]

We studied the cell killing effects of cisplatin (CDDP), 3′,5′-dichloromethotrexate (DCM), and different combinations of these drugs in a human colon carcinoma line (HCT-8). Using a clonogenic assay, the ED50 values for DCM were 3, 0.3, and 0.05 μM after 4-, 24-, and 48-hour exposure, respectively, while cell kill induced by CDDP was less time-dependent (ED50 values: 16, 7.8, 3.3, and 2.8 μM after 2-hour, 4-hour, 24-hour, and continuous incubation, respectively). Sequential exposure of these cells to DCM followed by CDDP resulted in synergism at every dose studied. The synergy was maximal with short DCM pretreatment intervals and decreased with increasing lengths of exposure to the antifol. In contrast, simple additive effects were observed when DCM was given together with or following CDDP. Considering the significant hepatic inactivation of both these compounds, our in vitro data encourage the design of clinical protocols with the sequence DCM → CDDP for hepatic arterial infusion treatment of liver metastasis from colon cancer. [Cancer Treat Rep 69:279–283, 1985]

The recent development of a totally implantable pump (1) designed for long-term arterial infusion of drugs has renewed the interest in those agents which are significantly inactivated by the liver. The administration of such agents directly into the hepatic artery may improve their therapeutic index by resulting in higher local drug concentration with a proportionally lower systemic toxicity.

Dichloromethotrexate (DCM) and cisplatin (CDDP) are potentially useful drugs for hepatic arterial infusion treatment of patients with metastasis due to gastrointestinal cancer (2,3). The antifol has long been known to undergo extensive degradation by hepatic aldehyde oxidase (4), while only recently, significant first-pass extraction of CDDP by the liver has been demonstrated as well (5). Because the combination of methotrexate and CDDP has shown synergistic tumor cell kill in vivo (6) and because DCM and CDDP are in clinical use (7), we studied the cell killing effect of the halogenated methotrexate analog alone and in different combinations with CDDP on the human colon tumor cell line HCT-8 prior to their use in patients with hepatic metastasis of colon carcinoma.

MATERIALS

CDDP was obtained from commercial sources and was the material available for clinical use. DCM was a gift from Lederle Laboratories and was > 95% pure as measured by high-pressure liquid chromatography.

METHODS

Tissue culture cells utilized in this investigation were from the continuously growing human colon adenocarcinoma line HCT-8 (8). Cells were maintained in 25-cm^2 sterile plastic flasks (Costar, Cambridge, MA) as monolayer cultures in RPMI-1640 medium (Grand Island Biological Co, Grand Island, NY) supplemented with 10% horse serum and subcultured weekly. Under these conditions, the doubling time was 18 hours.

Clonogenic Assay

A monolayer clonal growth technique was employed (9). Cell suspensions were obtained by trypsinization of stock cultures for 5 minutes. A high degree of monocel-

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TABLE 1.—% survival* of HCT-8 cells exposed to DCM, CDDP, and their combinations: 24-hr DCM, 2-hr CDDP

DCM (μM)	CDDP (μM)	DCM at Hours 1-24 and CDDP at Hours 22-24	DCM at Hours 1-24 and CDDP at Hours 1-2
0.5	0	26.1	26.1
2	0	11.9	11.9
0	30	38.0	38.0
0	60	10.5	10.5
0.5	30	6.5 (1.5)	12.0 (0.8)
0.5	60	1.5 (1.8)	4.1 (0.6)
2	30	3.0 (1.5)	5.0 (0.8)
2	60	0.6 (2.1)	1.8 (0.7)

* Results from 1 representative experiment. Mean values of 3 Petri dishes. SDs were always < 5%. Survival was measured by monolayer clonal growth technique and expressed as % of untreated controls (*see* Experimental Design). Nos. in parentheses indicate the coefficient of drug interaction (*see* Methods section).

lular dispersion was obtained by pipetting trypsinized cells followed by passages through needles of decreasing size (from 19 to 25 g). Portions of 400-2000 cells in 5 ml of medium containing 10% horse serum were pipetted into sterile 60-mm Petri dishes (Costar) and incubated at 37°C and 100% humidity, 7.5% CO_2. Eighteen hours later, when the cells were firmly attached to the bottom of the Petri dish, but had not yet divided, 0.1 ml of an appropriate dilution of drug in phosphate-buffered saline (PBS) (0.8% NaCl, 0.02% KCl, 0.115% Na_2HPO_4, and 0.02% KH_2PO_4) was added to each dish. Control dishes received the same volume of PBS. After the designed incubation period, the medium was decanted, the dishes were washed twice with 5 ml of PBS, and 5 ml of fresh medium was replaced. In sequential treatment experiments, this procedure was repeated after the exposure to the second drug. Ten days after the initial plating, colonies were stained with orcein and those colonies with > 30 cells were counted at ten times magnification using a dissecting microscope. Each experimental point was determined in triplicate with four replicate controls; experiments were repeated at least three times.

Experimental Design

Times and sequences of exposure to DCM and CDDP are shown in tables 1 and 2 and figure 1. In general, the doses selected for drug combination experiments were chosen to give a cell kill between 20% and 50%, so that maximum synergy could be observed. The quantitation of drug interaction was done by calculating the ratio between the product of the survival fraction of each individual drug and the survival fraction of the drug combination (10). Values > 1 were considered to show synergism; < 1, antagonism; and about 1, additive effect.

RESULTS

Cell Kill as a Function of Concentration and Time of Exposure to DCM and CDDP

HCT-8 cell kill produced by DCM and CDDP as a

TABLE 2.—% survival* of HCT-8 cells exposed to DCM, CDDP, and their combinations: 48-hr DCM, 2-hr CDDP

DCM (μM)	CDDP (μM)	DCM at Hours 1-48 and CDDP at Hours 46-48	DCM at Hours 1-48 and CDDP at Hours 1-2
0.05	0	45.0	45.0
0.1	0	24.8	24.8
0.2	0	14.1	14.1
0	30	17.5	17.5
0	60	5.1	5.1
0.05	30	10.3 (0.8)	8.2 (1.0)
0.05	60	2.1 (1.1)	2.3 (1.0)
0.1	30	3.9 (1.1)	5.4 (0.8)
0.1	60	1.0 (1.3)	1.6 (0.8)
0.2	30	2.1 (1.2)	3.4 (0.7)
0.2	60	0.5 (1.4)	1.0 (0.7)

* Results from 1 representative experiment. Mean values of 3 Petri dishes. SDs were always < 5%. Survival was measured by monolayer clonal growth technique and expressed as % of untreated controls (*see* Experimental Design). Nos. in parentheses indicate the coefficient of drug interaction (*see* Methods section).

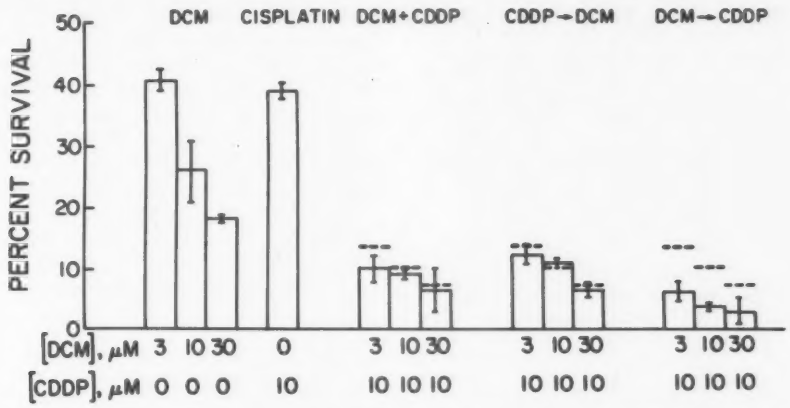

FIGURE 1.—Four-hr exposure of HCT-8 cells to DCM and CDDP: sequence-dependent effects on survival. Survival was evaluated by a monolayer clonal growth technique. Dashed lines indicate the expected survival times (*see* Methods section).

function of concentration and time of exposure is presented in figures 2 and 3. The ED50 value for DCM, defined as the concentration of drug killing 50% of cells compared to untreated controls, decreased by approximately one log, respectively, when HCT-8 cells were exposed to the antimetabolite for 4, 24, or 48 hours (3.0, 0.3, and 0.05 μM, respectively). Conversely, CDDP cytotoxicity was less time-dependent (ED50 values: 16, 7.8, 3.3, and 2.8 μM after 2-hour, 4-hour, 24-hour, and continuous incubation, respectively).

DCM-CDDP Combinations

Figure 1 shows the sequence-dependent effect of 4-hour incubation with DCM-CDDP combinations on survival of HCT-8 cells. Sequential exposure of these cells

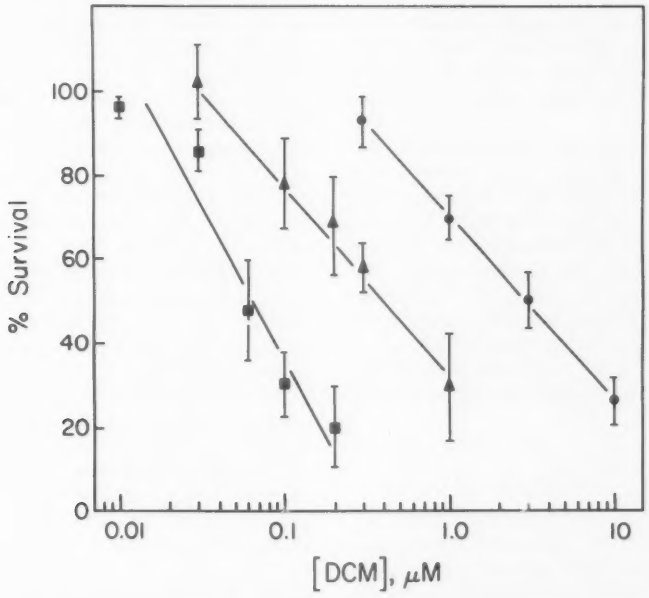

FIGURE 2.—Survival of HCT-8 cells exposed to DCM. ●—● = 4-hr, ▲—▲ = 24-hr, and ■—■ = 48-hr exposure. Survival was evaluated by a monolayer clonal growth technique.

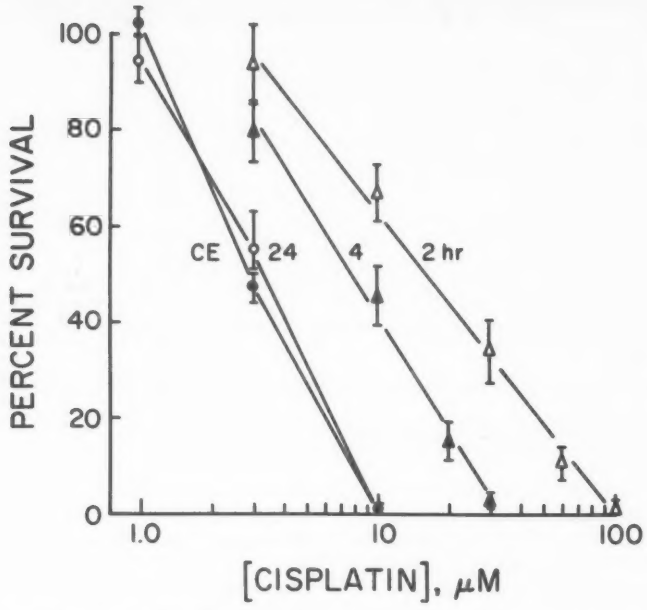

FIGURE 3.—Survival of HCT-8 cells exposed to CDDP. △–△ = 2-hr, ▲–▲ = 4-hr, ○–○ = 24-hr, and ●–● = continuous exposure. Survival was evaluated by a monolayer clonal growth technique.

to DCM (for 4 hours) followed by CDDP (for 4 hours) resulted in significant synergism at every dose studied (data with increasing concentrations of CDDP are not shown), while the sequence CDDP → DCM or the simultaneous exposure to the two agents gave only additive effect.

In an attempt to optimize the time schedule of administration of the two drugs, we have tested longer exposure to DCM (24–48 hours) with CDDP given for 2 hours either at the beginning or at the end of this incubation time (tables 1 and 2). When the brief exposure to CDDP was done during Hours 22–24 the synergy observed with shorter incubation with DCM → CDDP was still present, although less evident. The synergy was lost when CDDP was given during Hours 46–48.

The administration of CDDP at the beginning of the exposure to the antifol resulted in mild antagonism in both time schedules.

DISCUSSION

Our data clearly demonstrate synergistic tumor cell kill of the sequence DCM → CDDP on colon carcinoma cells in culture when the antifol precedes CDDP by up to 22 hours. This synergism may be explained by the effect of DCM pretreatment on the cell cycle kinetics of HCT-8 cells. The antimetabolite causes delay in progression and arrest of cells at the G_1/S boundary (11); the subsequent administration of CDDP would result in increased lethality of this drug, since cellular DNA crosslinking by platinum complexes is maximal after exposure during G_1 phase (12). With longer DCM incubation, the simple additive effect observed between the drugs may be accounted for by the actual cell death during S phase induced by the antifol and the concomitant reduction of the expanded pool of cells in G_1. In addition, the effect of dihydrofolate reductase inhibition by DCM on RNA, protein, and repair-related DNA synthesis (13) may inhibit the repair of CDDP-induced DNA injury resulting in irreversible cell damage. This process of "complementary inhibition" between drugs (14) may also explain the observed time schedule-dependent synergy.

Methotrexate (MTX) and CDDP in combination have displayed additive and synergistic cell kill in in vitro (15) and in vivo (6,16) experimental tumor systems, but this two-drug combination has not been used widely in the clinic because of potential combined nephrotoxicity and impairment of MTX renal excretion by CDDP. While MTX and CDDP, with or without bleomycin, have been used to treat patients with head and neck cancer, MTX has usually been administered 1 day to 1 week preceding CDDP to avoid renal toxicity (17). The metabolizable MTX analog, DCM, should not have the

disadvantage of the parent compound, and this drug combination is in clinical trial for systemic treatment of head and neck, bladder, and cervical cancers (7). Further studies are needed to clarify whether the sequence-dependent synergy observed on the human colon carcinoma line HCT-8 is a general phenomenon; nevertheless, since the two drugs can be easily administered via the implantable pump and are both significantly inactivated by the liver, our in vitro data encourage the design of clinical protocol with the sequence DCM → CDDP for hepatic arterial infusion treatment of liver metastasis from colorectal cancer.

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Effect of Forphenicinol, a Small Molecular Immunomodifier, in Combination With Cyclophosphamide on Growth of and Immunity to Syngeneic Murine Tumors[1,2]

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Forphenicinol (FPL) is a low molecular immunomodifier derived from forphenicine, a microbial product found by Umezawa and co-workers. We studied the antitumor effect of FPL, cyclophosphamide (CY), and the combination of the two on several syngeneic murine tumors. The tumors used were mammary carcinoma, L1210 leukemia, B16 melanoma, Lewis lung carcinoma, and glioblastoma. A single ip injection of CY on Day 1 followed by eight consecutive daily oral doses of FPL beginning 6 days after tumor inoculation showed strong cooperation in curing syngeneic mammary carcinoma inoculated intradermally in C3H/HeN mice, most mice being cured of the tumor by the combination therapy and subsequently having acquired strong specific immunity. Treatment with FPL alone (either pre- or post-treatment) also significantly inhibited the growth of the mammary tumor. FPL and CY also showed cooperation in inhibiting the growth of L1210 leukemia transplanted intradermally into CDF_1 (BALB/c × DBA/2) mice and markedly prolonged the survival time but FPL treatment alone had no effect. The FPL-CY treatment also affected Lewis lung carcinoma and glioblastoma in syngeneic C57BL/6 mice and produced therapeutic synergism. FPL alone significantly inhibited the growth of B16 melanoma in C57BL/6 mice as well as the syngeneic mammary carcinoma in C3H/HeN mice. These findings suggest that oral administration of FPL in combination with chemotherapeutic agents can be used for treating cancer without causing toxicity, because of the synergistic efficacy of the combination. [Cancer Treat Rep 69:285–291, 1985]

Research on antibiotics has demonstrated that microorganisms can produce an enormous number of compounds which have various structures. In 1965, Aoyagi et al (1) and Umezawa (2) screened microbial culture broths for enzyme inhibitors of small molecular weight and found nearly 40 enzyme inhibitors. Forphenicine (FPN) (fig 1) is one of these compounds which was found to be an inhibitor of chicken intestine alkaline phosphatase in the culture filtrate of *Actinomyces fulvoviridis* var. *acarbodicus*. Forphenicinol (FPL) (fig 1) was derived from FPN and has immune-modifying activity but no mitogenicity (3). The toxicity of FPL is very low in various animals by any route of administration (4). The mechanism of FPL-mediated enhancement of the immune response is postulated to be activation of macrophages, since oral administration of FPL restores delayed type hypersensitivity in immune-suppressed mice and enhances phagocytosis of peritoneal macrophages in vivo and in vitro. FPL neither augments antibody formation nor stimulates proliferation of lymphocytes in the presence or absence of lectins. FPL also increases granulocyte-macrophage colony-forming unit production in bone marrow cell cultures in the presence of colony-stimulating factor (3). The present work was undertaken to determine the effect of FPL in combination with cyclophosphamide (CY) on tumor growth or tumor immunity of several syngeneic murine tumors, and the results were compared with those of FPL or CY treatment alone.

MATERIALS AND METHODS

Animals.—Young adult syngeneic mice (female C3H/

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FIGURE 1.—Structures of FPN and FPL.

HeN, male C57BL/6, and male CDF_1 [semisyngeneic]) were used. We also used nu/nu C3H/HeN mice that were bred in our laboratory (parents were supplied by Steering and Program Coordinating Group for the Co-operative Cancer Research, Ministry of Education, Science and Culture). In general, each group consisted of six mice.

Tumors.—The syngeneic mammary tumor line 080679, used at the 25th transplantation, had been spontaneously generated in a C3H/HeN mouse and maintained by sc transplantation. Mice began dying about 30 days after intradermal inoculation with 1×10^6 cells. Other tumors used were L1210 leukemia, B16 melanoma, Lewis lung carcinoma (LLC), and glioblastoma (5), which were maintained in our laboratory in syngeneic mice. Except for L1210, these tumors were treated with enzymes as described by Rapp et al (6) to make a single-cell suspension, and viable cells ($1-2 \times 10^6$) were inoculated intradermally into the hindquarters of mice on Day 0. Briefly, 3 ml of 0.04% deoxyribonuclease (Worthington Biochemicals Corp, Freehold, NJ) in saline was added to 100 ml of 0.25% pronase E (Kaken Seiyaku Co, Ltd, Tokyo, Japan) in Hanks' balanced salt solution (HBSS). Finely minced tumor tissue was placed in a trypsinizing flask; 10 ml of the enzyme solution/1 g of tumor tissue was added and mixed at room temperature with a magnetic stirrer for 10 minutes; and the supernatant fluid was then decanted. Digestion of the tumor tissue by this procedure was repeated until sufficient cells were obtained. The cells were washed twice in HBSS and resuspended in medium 199. Viable cells were counted by means of the trypan blue exclusion test. For the inoculum of L1210, the ascites tumor cells were used.

Forphenicinol and cyclophosphamide.—FPL was chemically synthesized (7). It was dissolved in distilled water and administered orally in various doses five to eight times after tumor inoculation or two to three times before tumor inoculation. A saline solution of CY (Shionogi Co, Ltd, Osaka, Japan) was prepared just before use and injected ip on Day 1 (on Day -4 in the pretreatment experiment) in doses varying from 25 to 200 mg/kg as indicated for each experiment.

Latex particles.—A suspension of uniform polystyrene particles (1.0 μm in diameter), obtained from the Dow Chemical Co, Indianapolis, IN. was sterilized at 65°C for 40 minutes and mice were injected ip at a dose of 60 mg/adult mouse (8).

Measurement of tumors and test for tumor immunity.—Intradermal tumors in mice were measured weekly; tumor size was measured as two diameters at right angles and their product (length × width) and dates of death of the animals were recorded. In each experiment, when mice began dying, the survivors that did not develop tumors because of the treatment were challenged intradermally with the tumor cells at a site contralateral to the first tumor inoculation. The animals were examined for acceptance of the tumor, ie, the overall assessment of therapy was based on the rate of tumor growth inhibition, the rate of tumor-free animals at the time of tumor challenge, and induction of tumor immunity after the tumor challenge. In experiments with L1210, the assessment was made by the survival time.

RESULTS

Effect of FPL Combined With CY on Growth of and Immunity to Mammary Tumor in C3H/HeN Mice (tables 1 and 2)

Female mice were inoculated with viable 1×10^6 cells of the mammary carcinoma, and 1 day after the inoculation some of them received an ip injection of CY at a dose of 50 or 25 mg/kg. As seen in table 1, mice in two of three groups at each dose of CY were treated orally with 0.2 ml of FPL solution at 0.5 or 0.1 mg/kg. Thirty days after the tumor inoculation, mice that rejected the tumor were

TABLE 1.—Effect of Treatment with FPL in combination with CY on growth of and immunity to mammary tumor in C3H/HeN mice

Group No.	Dose (mg/kg/day)		No. of mice	Tumor size on Day 29 (mm^2 ± SD)*	Inhibition ratio (%)†	Tumor challenge on Day 30‡	No. of immunized mice/ No. challenged§
	CY, ip, Day 1	FPL, oral, Days 6–13					
1	50	—	6	145 ± 18	34	1/5	0/1
2	50	0.5	6	95 ± 99	57	4/6	4/4
3	50	0.1	6	63	71	5/6	5/5
4	25	—	6	162 ± 49	26	0/5	—
5	25	0.5	6	70 ± 67	68	3/6	2/3
6	25	0.1	6	61 ± 16	72	4/6	3/4
7	—	0.5	6	117 ± 47	47	0/5	—
8	—	—	4	220 ± 22	—	0/3	—
9	Challenge control	—	7	—	—	—	—

* Tumor-free mice are excluded.
†% inhibition of tumor growth (1 - treated/control) × 100.
‡Tumor-free mice that received tumor challenge (1.5×10^6 cells) on Day 30/No. of mice that survived 30 days.
§Evaluation was made on Day 51.

challenged with 1.5×10^6 tumor cells of the same line. The results can be summarized as follows. Mice treated with CY or FPL alone showed moderate tumor suppression ($P < 0.05$) but no effect on tumor immunity. In comparison, mice treated with CY and FPL showed strong suppression of the tumor growth ($0.01 > P > 0.001$) and developed tumor immunity at a high rate (14 of 16 mice). In this experiment, specificity of the tumor immunity was ascertained by inoculation of a different syngeneic mammary tumor line (073082, another spontaneous mammary carcinoma developed in a C3H/HeN mouse) into survivors of the challenge inoculation of mammary carcinoma line 080679 (table 2).

Effect of FPL Combined With CY on the Survival of L1210-Bearing CDF_1 Mice (fig 2)

Male CDF_1 mice were inoculated intradermally with 1×10^6 L1210 cells, and FPL was given ip at various daily doses twice before and five times after tumor inoculation. CY was injected ip at a dose of 100 mg/kg on Day 1. As shown in figure 2, treatment with CY alone prolonged the survival time of mice bearing L1210, but FPL alone did not have any effect on the survival time. FPL at a dose of 0.01 mg/kg with CY treatment significantly enhanced the effect of CY, the % ILS [increase in median lifespan; calculated by (T/C - 1) × 100] being > 367%.

TABLE 2.—Induction of specific immunity by treatment with FPL or FPL + CY in C3H/HeN mice bearing syngeneic mammary tumor

Previous treatment		Rechallenge tumor*	
		Tumor size on Day 77, length × width (mm^2)	
CY, ip, on Day—	FPL, oral, 0.1 mg/kg/day on Days—	Line 080679	Line 073082
-4 (25 mg/kg)	-3 to -1	0	210
—	-3 to -1	12	168
1 (50 mg/kg)	6–13	0	180
—	6–13	0	345
Rechallenge control		225	150

* Primary inoculation (intradermal): 1×10^6 cells of line 080679 (32TG) on Day 0. Rechallenge (intradermal): 1.2×10^6 cells of line 080679 in the left back and 1×10^6 cells of another syngeneic mammary carcinoma line 073082 (1TG) in the right back on Day 40.

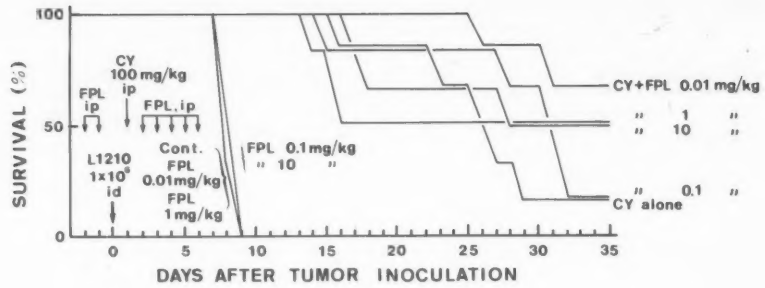


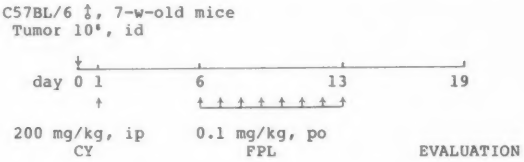
FIGURE 2.—Effect of FPL in combination with CY on survival time of CDF_1 mice inoculated intradermally with L1210 tumor cells.

ILSs of the groups of CY alone, CY plus FPL at 0.1 mg/kg, CY plus FPL at 1 mg/kg, and CY plus FPL at 10 mg/kg were 247%, 313%, 113%, and 273%, respectively. We terminated the experiment on Day 35 because all survivors on Day 33 were tumor-free. All of the mice that died by Day 32 carried tumors.

Effect of FPL Combined With CY on Growth of B16 Melanoma, LLC, and Glioblastoma (tables 3-5)

The effect of FPL in combination with CY on three syngeneic tumors in C57BL/6 mice was determined. As shown in table 3, mice were inoculated intradermally with 1×10^6 B16 melanoma cells and given a single ip injection of CY on Day 1 (200 mg/kg) followed by eight consecutive daily doses of FPL (0.1 mg/kg) on Days 6-13. CY or FPL alone resulted in significant tumor suppression ($P < 0.001$) and FPL plus CY also suppressed tumor growth ($P < 0.001$). There were tumor-free mice in Groups 2 and 4 on Day 19 (table 3), and when they were challenged intradermally with 1.2×10^6 tumor cells, none of them rejected the tumor challenge. A similar protocol of FPL-CY treatment was used for LLC. As seen in table 4, some synergism between the two agents was ob-

TABLE 3. Effect of FPL in combination with CY on growth of B16 melanoma



Group	CY 200 mg/kg ip, on day 1	FPL 0.1 mg/kg po	Tumor size (mm² ± SD)	% Inhib.	No. of tumor-free mice	t-Test vs.Gr.1	t-Test Gr.2	t-Test Gr.3
1	-	-	309 ± 30[a)]	--	0/6	--	--	--
2	+	-	133 ± 47	57	3/6	***	--	--
3	-	+, day 6 -x 8	146 ± 53	53	0/6	***	--	--
4	+	+, day 6 -x 8	44 ± 15	86	1/6	***	**	**

Animal: C57BL/6 ♀, 7-week old mice, 6 mice per group
Tumor: 1 x 10^6 cells id on day 0
Evaluation: on day 19
**: highly significant, $0.001 < p < 0.01$;
***: very highly significant, $p < 0.001$

a) Tumor-free mice are excluded.

TABLE 4.—Effect of FPL in combination with CY on growth of LLC*

Group No.	Dose (mg/kg)		Tumor size ($mm^2 \pm SD$)		% inhibition	t test vs—	
	CY, ip, Day 1	FPL, oral, Days 6–13	Day 22	Day 24		Group 1	Group 2
			Experiment 1				
1	—	—	530 ± 121		—	—	
2	50	—	415 ± 72		22	$0.10 > P > 0.05$	—
3	50	0.02	303 ± 90		43	$0.005 > P > 0.002$†	$0.05 > P > 0.02$‡
4	50	0.1	316 ± 60		40	$0.005 > P > 0.002$†	$0.05 > P > 0.02$‡
5	50	0.5	282 ± 75		47	$0.002 > P > 0.001$†	$0.02 > P > 0.01$‡
			Experiment 2				
1	—	—		564 ± 99	—	—	—
2	—	0.02		494 ± 165	12	—	—
3	—	0.1		411 ± 57	27	$0.50 > P > 0.20$	—
4	—	0.5		502 ± 108	11	—	—
5	—	2.5		482 ± 99	15	—	—

* Animal: C57BL/6 male, 6-wk-old mice, 6/group. Tumor: 1×10^6 cells intradermally on Day 0.
†Highly significant.
‡Significant.

served in the group treated with FPL and CY. Inhibition of tumor growth by CY treatment (50 mg/kg ip) was 22%, while that by CY with FPL at various doses ranged from 40% to 47%, with $0.05 > P > 0.02$ against the CY group. Treatment with FPL alone at doses of 0.02–2.5 mg/kg did not have much effect on tumor suppression (table 4, Experiment 2). Using the same protocol, the effect of the FPL and CY combination on glioblastoma was studied. Mice were inoculated intradermally with 2×10^6 cells on Day 0 and CY on Day 1 (100 mg/kg ip) followed by daily administration of FPL (0.1 mg/kg orally) on Days 6–13. As shown in table 5, neither CY nor FPL alone had any significant inhibiting effect on tumor growth, but FPL combined with CY had a significant effect on tumor suppression ($P < 0.05$). There were no tumor-free mice in the experiment with either LLC or glioblastoma.

Effect of FPL on Growth of Mammary Tumor in Nude and Normal C3H/HeN Mice

We determined whether the effect of FPL on mammary tumor would be altered in T-cell-deprived C3H/HeN mice. As shown in table 6, FPL treatment markedly inhibited the tumor growth in normal mice, but in nu/nu mice the inhibiting effect was completely nullified. In nu/+ mice and in mice treated with an antimacrophage agent (latex particles), the inhibitory effect of FPL was reduced by one-half.

DISCUSSION

FPL, a small molecular immunomodifier, is of great interest, since this compound has been reported to enhance the immune responses and to exert an antitumor effect in combination with 6-mercaptopurine, aclarubicin, or CY in several murine tumors (4). A characteristic of FPL is that it has extremely low toxicity in various animals by any route of administration (3,4). These findings led us to examine the antitumor effect of FPL combined with CY therapy on several syngeneic mouse tumor systems, such as mammary carcinoma, L1210 leukemia, B16 melanoma, LLC, and glioblastoma.

CY is an agent with one of the broadest antitumor spectra in cancer chemotherapy. Moreover, CY was chosen because its effectiveness in tumor immunity in combination with bacillus Calmette-Guérin (BCG) presensitization was shown in our previous experiments (9,10). The BCG-CY therapy also protected animals from the immunosuppressive effect of CY. Various chemotherapeutic agents other than CY (ie, doxorubicin, tegafur, aclarubicin, and busulfan) exhibited no cooperation with BCG presensitization in therapeutic efficacy (unpublished data). We think that FPL is a hopeful candidate for chemoimmunotherapy of cancer in combination with CY. The final goal of our experimental studies is to establish

TABLE 5.—Effect of FPL in combination with CY on growth of glioblastoma*

Group No.	CY, ip, 100 mg/kg, Day 1	FPL, oral, 0.1 mg/kg, Days 6–13	Tumor size on Day 35 (mm^3 ± SD)	% inhibition	t test
1	-	-	196 ± 56	—	—
2	+	-	146 ± 55	26	NS†
3	-	+	187 ± 78	5	NS†
4	+	+	114 ± 56	42	$P < 0.05$‡

* Animal: C57BL/6 male, 7-wk-old mice, 5/group. Tumor: 2×10^6 cells intradermally on Day 0.
†NS = not significant.
‡Significant.

optimal protocols of chemoimmunotherapy to cure solid cancer and to prevent spontaneous metastasis and recurrence of cancer which will serve as a model for clinical use. Therefore, in the present study, we designed a protocol to determine the efficacy of FPL combined with CY for solid tumors in syngeneic mice. Before the experiments, the optimal dosage of CY in each strain system was determined (50 mg/kg in C3H/HeN mice and 150–200 mg/kg in C57BL/6 mice).

Against the mammary tumor and B16 melanoma, the treatment with FPL alone caused significant inhibition of tumor growth, $P < 0.05$ and $P < 0.001$, respectively (table 1, Group 7 and table 3, Group 3). Treatment with FPL with CY induced a very high incidence of specific tumor immunity in the mammary tumor system as well as tumor suppression (table 1, Groups 2, 3, 5, and 6). In all tumor systems we tested, the FPL-CY combination therapy exhibited therapeutic synergism in comparison with the efficacy of either agent alone (fig 2 for L1210, table 4 for LLC, and table 5 for glioblastoma). In mice bearing a fast-growing tumor such as L1210 in solid form, administration of FPL (orally) by itself did not affect their survival time, but the combination of FPL and CY (ip) did markedly prolong their survival time (fig 2).

The mechanism of antitumor effect of FPL is not yet clear, but the enhancement of the effect of CY by FPL may be due to macrophage activation and restoration of the defense mechanism damaged by CY (3). T cells were shown to be necessary for the antitumor effect of FPL by our present nude mouse experiment. It is interesting to note that the inhibitory effect of FPL in nu/+ mice was reduced by one-half. A few investigators described the possibility of discriminating heterozygous (nu/+) mice from wild type homozygous (+/+) mice by thymic weight, peripheral leukocyte count, stem cell potentiation in bone marrow, and in some immune responses (11–13). Nevertheless, these efforts have not been widely approved and many investigators still use heterozygotes as normal control. It is necessary to clarify the precise phenotypic difference between these two groups (C3H/HeN nu/+ and +/+ mice).

The participation of macrophages was also demonstrated by the use of an antimacrophage agent. We recently obtained similar results by repeating the experiment with antimacrophage agents. For the antitumor effect of FPL, we assume that the induction of effector T cells caused by the activated macrophages is very important. In the course of treatment with CY in combination with FPL, it is necessary to clarify the underlying mechanism of their immunological interaction in vivo. At any rate, FPL therapy in combination with anticancer drugs is expected to be a very hopeful and important treatment against cancer. Further studies on the mechanism of action of the combination therapy and studies to find a

TABLE 6.—Effect of oral administration of FPL on the growth of syngeneic mammary tumor in nude, macrophage-suppressed, and normal mice*

Group No.	FPL, oral, 0.1 mg/kg, Day—	Latex particles, 60 mg/mouse, ip, Day -5	Type of mice	% inhibition of tumor growth†
1	-3 to -1	-	Normal	55
2	-3 to -1	+	Normal	27
3	-3 to -1	-	nu/nu	-17
4	-3 to -1	-	nu/+	22
5	6–13	-	Normal	45
6	6–13	-	nu/nu	-27
7	6–13	-	nu/+	23

* Mice: female CRJ-C3H/HeN, 12–13-wk-old mice for normal mice; nu/nu and nu/+ mice of C3H/HeN were bred in our laboratory; 6/group. Tumor: line 080679 mammary carcinoma, 43rd transplant generation; 1×10^6 cells intradermally on Day 0.
†Evaluation: on Day 36.

model for clinical therapeutic efficacy against cancer are underway.

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Arterial Drug Infusion With Extracorporeal Removal. II. Internal Carotid Carmustine in the Rhesus Monkey[1]

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During cancer chemotherapy by intra-arterial drug administration, systemic toxicity often limits the tolerable dose. We evaluated the pharmacokinetic advantage obtained by infusing carmustine (BCNU) into the internal carotid artery during BCNU removal from the blood from the perfused region by hemoperfusion. A hemoperfusion column (XR-010, Extracorporeal Medical Specialties) was shown to remove BCNU quantitatively from sheep blood flowing at 300 ml/minute when the drug was infused at 13 mg/minute for 30 minutes. Under general anesthesia, adult rhesus monkeys underwent catheterization of the internal carotid artery and placement of a catheter in the ipsilateral jugular vein at its junction with the sigmoid sinus. BCNU (10 mg/kg) was infused over 20 minutes while blood was pumped from the jugular vein through a small column and back into the inferior vena cava. The procedure reduced systemic exposure by 46%–84% compared with iv infusion of the same dose. Brain-to-systemic exposure ratios ranged from 18:1 to 87:1, depending on the pump flow rate and method of calculation. Hematopoietic toxicity was prevented. It is suggested that tumor exposure to BCNU comparable to that associated with very high tumor cell kill in vitro may be feasible with little or no systemic toxicity. [Cancer Treat Rep 69:293–303, 1985]

During cancer chemotherapy, toxicity to tissues other than those in which the tumor resides frequently limits the tolerable dose. Marrow suppression, renal damage, or toxicity to peripheral nerves or cardiac muscle frequently restricts the dose and frequency of drug administration with a concomitant reduction in tumor response. Procedures which significantly increase the ratio of tumor exposure to systemic exposure of an active drug should yield therapeutic advantage. Intra-arterial therapy with an appropriate agent is based on this principle.

Pharmacokinetic analyses have established that, if the system is linear and the perfused region does not eliminate a significant amount of drug, the advantage of arterial delivery is a function only of local arterial flow rate and total-body clearance (1–3). For a given artery a therapeutic advantage may be obtained by selection of an active drug with a high total-body clearance or by the application of a technique which increases total-body clearance.

The nitrosoureas, carmustine (BCNU) and lomustine (CCNU), have proven activity in the treatment of patients with malignant brain tumors (4). However, their clinical effect is modest, and the tumors are uniformly fatal. Since gliomas produce morbidity and mortality by local growth and rarely metastasize, they are suitable for regional therapy. Internal carotid artery (ica) administration of BCNU is now being used to treat these tumors, and early reports suggest an improved response rate (5–7). In rhesus monkeys, despite increased delivery of BCNU to the brain by ica administration, systemic toxicity limits the tolerable dose (8). We sought to increase the pharmacokinetic advantage of intra-arterial infusion by combining it with drug removal from the perfused region's venous drainage by hemoperfusion.

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The purpose of this study was to evaluate the pharmacokinetic advantage obtained by infusing BCNU into the ica during drug extraction from the ipsilateral jugular drainage. We also compared the hematopoietic toxicity of this procedure with that produced by iv injection of the same dose. Although we limited this study to the brain, the same techniques and pharmacokinetic principles could be applied to any organ in which the regional vascular anatomy is appropriate for intra-arterial drug delivery and for collection of the venous drainage for drug removal in an extracorporeal device.

MATERIALS AND METHODS

Materials

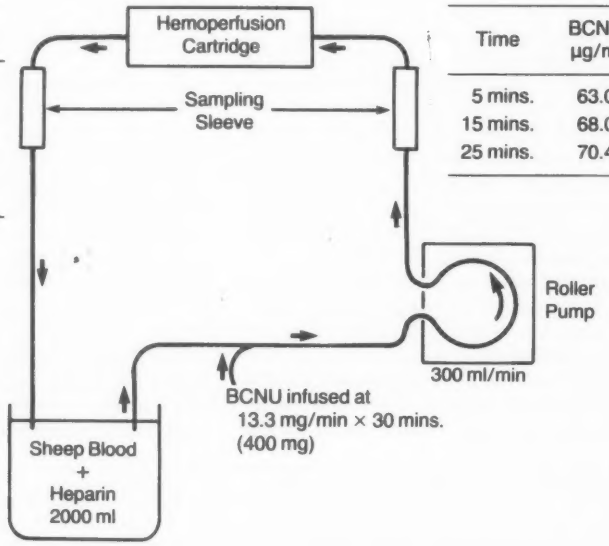
BCNU was obtained from the Drug Development Branch, National Cancer Institute (Bethesda, MD) and mixed with absolute ethanol and sterile water immediately before administration to yield a concentration of 3.3 mg/ml of BCNU in 10% alcohol.

In the in vitro and in vivo experiments blood was pumped through a hemoperfusion cartridge containing a nonionic adsorbent resin, which is a copolymer of polystyrene and divinyl benzene (Amberlite XAD-4 resin, registered trademark of Rohm and Haas Co). Associated blood lines and instrumentation are shown in figures 1 and 2. The in vitro experiment was performed with an extracorporeal clinical cartridge containing approximately 310 g dry weight of resin (XR-010 Hemperfusion System, Extracorporeal Medical Specialties, Inc, King of Prussia, PA). Because of the monkey's small blood volume, we used a cartridge containing about 100 g of resin and a specially designed tubing set to limit the priming volume to approximately 240 ml. A roller pump (Sarns, Inc, Ann Arbor, MI) was used to propel the blood through the hemoperfusion circuit.

BCNU Assay

Analysis of plasma samples for BCNU content was performed by means of high-performance liquid chromatography (9). Whole blood samples were cooled in an ice bath immediately after being drawn. They were then centrifuged at 2000 × *g* for 8 minutes, plasma was removed (0.5–1.0 ml), pH was adjusted to 4 with 4% (vol/vol) glacial acetic acid, and samples were frozen until analysis. Standards were made in fresh acidified plasma. Propyl paraben was added as an internal standard. Samples and standards were then extracted with 1.25% (vol/vol) ethyl alcohol in diethyl ether. The extracts were separated, evaporated to dryness, and refrigerated until analysis. Chromatography was performed using a reverse-phase C-18 analytical column preceded by a guard

Time	BCNU µg/ml
5 mins.	<0.05
10 mins.	<0.05
15 mins.	<0.05
20 mins.	<0.05
25 mins.	<0.05


Hemoperfusion Cartridge
Sampling Sleeve
Roller Pump
300 ml/min
BCNU infused at 13.3 mg/min × 30 mins. (400 mg)
Sheep Blood + Heparin 2000 ml

Time	BCNU µg/ml
5 mins.	63.0
15 mins.	68.0
25 mins.	70.4

FIGURE 1.—Schematic of in vitro circuit. Heparinized sheep whole blood was pumped through the circuit at 300 ml/min while BCNU was infused into the cartridge inflow line. Blood from the cartridge inflow and outflow lines was sampled for determination of plasma BCNU concentrations at intervals after beginning BCNU infusion. No BCNU was detected in the plasma leaving the cartridge.

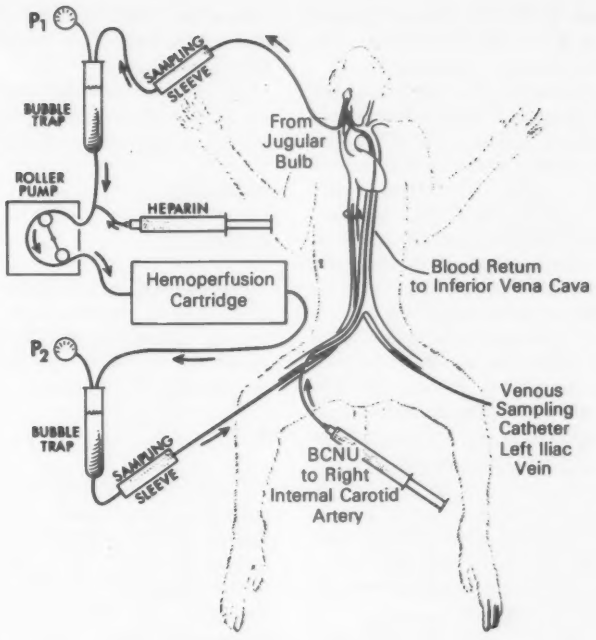


FIGURE 2.—Extracorporeal circuit and catheter placement in treatment combining hemoperfusion of jugular blood with intracarotid infusion of BCNU. Blood was propelled through the hemoperfusion system at either 30 or 60 ml/min by a roller pump while 10 mg/kg of BCNU was infused into the ica over 20 mins.

column. The mobile phase consisted of 35% (vol/vol) acetonitrile and 0.1% glacial acetic acid in water, pumped at 1.2 ml/minute. Detection was by ultraviolet absorbance at 230 nm. Concentrations were determined by comparison of peak height ratios (BCNU/internal standard analyzed by weighted least-squares linear regression [weight = 1/(measured concentration)^2]. The sensitivity of the assay is 0.050 μg/ml (0.025 μg/ml if 1.0 ml of plasma is used).

In Vitro Experiment

We initially determined the BCNU-removing capability of the cartridge in vitro (fig 1). Ten thousand units of heparin was added to 2000 ml of whole sheep blood at 32°C. The blood was pumped at 300 ml/minute through a circuit containing the hemoperfusion cartridge while 400 mg of BCNU was infused over 30 minutes into the cartridge inflow line. After the infusion began, samples for plasma BCNU determination were obtained at 5, 15, and 25 minutes from the blood entering the cartridge and at 5-minute intervals for 30 minutes from the blood leaving the cartridge.

In Vivo Experiment

Adult rhesus monkeys were fasted overnight and then anesthetized with iv pentobarbital. Catheters were placed in the right ica, the inferior vena cava, the left iliac vein, and the right jugular bulb (fig 2).

For BCNU infusion the right ica was catheterized transfemorally with a polyethylene catheter (internal diameter, 0.037 inches) via a 2-cm right groin incision. Carotid arteriography was then performed to confirm the position of the catheter tip within the ica and to ensure unimpeded carotid blood flow. For blood return to the animal from the extracorporeal system, a second polyethylene catheter (internal diameter, 0.067 inches) was positioned through the same groin incision into the right femoral vein so that the catheter tip lay in the inferior vena cava just below the level of the diaphragm. The left femoral vein was catheterized and the catheter positioned in the left iliac vein. This was used for sampling blood for systemic BCNU concentrations. A fourth catheter carried the jugular blood extracorporeally into the hemoperfusion system. A 1½-cm cervical incision was made and an 8-Fr pediatric feeding catheter was ad-

vanced through a venotomy in the right internal jugular vein until the catheter tip lay in the jugular bulb. This position was confirmed by retrograde venography.

The monkey was then given an iv pulse injection of 800 units of heparin. The extracorporeal system was primed with normal saline containing 1500 units/L of heparin. This priming solution was mostly displaced by 200 ml of pooled rhesus whole blood to which 750 units of heparin had been added. The hemoperfusion system was connected to the jugular and inferior vena caval catheters, and the jugular blood was pumped extracorporeally through the hemoperfusion system and back into the inferior vena cava at a rate of 30 or 60 ml/minute by a roller pump (fig 2). During hemoperfusion heparin was infused into the cartridge inflow line at 300 units/hour. When the extracorporeal circuit was flowing smoothly we began the BCNU infusion (10 mg/kg infused over 20 minutes). Continuous infusion of BCNU and heparin was by a syringe infusion pump (Harvard Apparatus Co, Inc, Dover, MA). Blood samples were obtained frequently from the cartridge inflow and outflow lines and from the left iliac vein during 180 minutes of hemoperfusion after beginning the BCNU infusion. The monkey's neurological status was monitored immediately after the procedure, daily for 4–5 days, and weekly thereafter.

Six to 9 weeks following the procedure described above we infused BCNU (10 mg/kg) iv over 20 minutes and sampled blood from the iliac vein for BCNU measurement on a schedule similar to that used during the combined treatment.

Cbcs, platelet counts, and electrolytes were determined before each treatment and at weekly intervals for at least 6 weeks after each treatment.

Mathematical Methods

We calculated the brain:body exposure ratio for each animal during the combined treatment using two independent methods. In one method, the brain exposure was calculated assuming ica blood flow of 20 ml/minute (based on supratentorial brain weight of 80 g and cerebral blood flow of 50 ml/100 g/minute) (10–12) and the systemic exposure as discussed below. For the second method we used the formula

$$\frac{AUC_{brain}}{AUC_{body}} = \frac{1 + \frac{EQ_s + k_2V_2}{Q}}{1\text{-}fE},$$

where E is the fraction of drug removed from the blood flowing through the cartridge, Q is the ica flow rate (20 ml/minute), f is the fraction of blood into which the BCNU is infused which is collected for extracorporeal circulation, Q_s is the flow through the cartridge from the rest of the body (ie, blood flowing through the hemoperfusion circuit in addition to the right ica blood flow receiving the BCNU infusion), and k_2V_2 is the BCNU clearance by the body. This formula is discussed in detail by Dedrick et al (13).

The systemic exposure was calculated by determining the AUC by the trapezoidal method with extrapolation to infinity using an assumed BCNU half-life of 40 minutes.

RESULTS

In Vitro

Plasma BCNU concentrations entering the cartridge at 5, 15, and 25 minutes after beginning BCNU infusion into the inflow line were 63.0, 68.0, and 70.4 μg/ml, respectively. No BCNU was detected in the samples from the cartridge outflow (fig 1).

In Vivo

Five monkeys received ica BCNU combined with hemoperfusion. Two animals became hypotensive and hypothermic during extracorporeal circulation. One monkey recovered slowly from anesthesia and had no focal neurological deficit the evening of the procedure but died that night. The other animal could not be resuscitated. Gross inspection of these monkeys' brains revealed no evidence of focal or diffuse abnormality. One of the remaining three animals (No. T480) awoke from the combined treatment with mild weakness of the left leg. This resolved fully within 18 hours. One monkey (No. 7614) had left hemiparesis during the combined treatment. This gradually disappeared over 3 weeks. There was no other evidence of neurological deficit. All animals experienced acute but transient chemosis and erythema of the eye ipsilateral to the carotid infusion. There was no evidence of persisting ocular damage. All of the animals experienced significant thrombocytopenia (platelet counts, 21,000–31,000/mm^3) during hemoperfusion.

The combination of intracarotid BCNU infusion and hemoperfusion of the ipsilateral jugular blood increased total-body BCNU clearance (Cl_{tb}), decreased systemic BCNU exposure (AUC_{body}), increased brain BCNU exposure (AUC_{brain}), and reduced hematopoietic toxicity compared to iv administration of the same dose. Assuming ica flow of 20 ml/minute, the combined treatment increased the ratio of brain BCNU exposure to systemic BCNU exposure by 18–69-fold. Calculations based on an independent pharmacokinetic analysis of

$$\frac{AUC_{brain}}{AUC_{body}},$$

using the previously cited formula, resulted in brain-to-systemic BCNU exposure ratios of 30–87 to 1. Thus, there was general concordance of the pharmacokinetic advantage determined by using the two different

TABLE 1.—Pharmacokinetics of hemoperfusion during intracarotid BCNU infusion compared to iv BCNU infusion

Monkey No.	Weight (kg)	Flow through cartridge (ml/mm)	BCNU (mg/kg)	Infusion (iv vs ica + hemoperfusion)	Exposure to BCNU (μg-min/ml)		Cl_{tb} (ml/min/kg)	Pharmacokinetic advantage*	
					Systemic	Brain†		Ica† (20 ml/min)	Independent pharmacokinetic calculations†
1172	11.7		10	Iv	648	648	15.4	1	1
		30	10	Ica + hemoperfusion	347	6197	28.8	18	30
T480	9.7		10	Iv	472	472	21.2	1	1
		59	10	Ica + hemoperfusion	120	4770	83.3	40	39
7614	9.0		10	Iv	421	421	23.8	1	1
		60	10	Ica + hemoperfusion	66	4566	151.5	69	87

* $\frac{AUC_{brain}}{AUC_{body}}$. Exposure to BCNU for the body is AUC.

†See text.

methods of calculation (table 1).

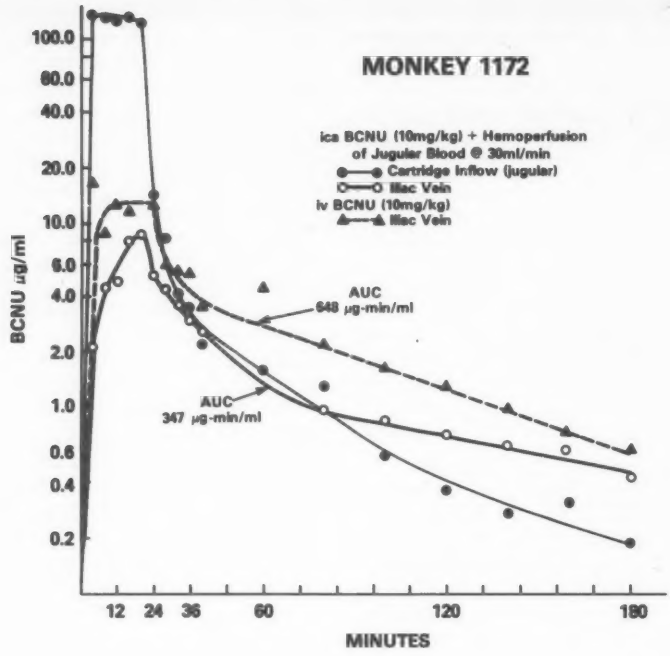
After iv administration the Cl_{tb} was 20 ± 4 ml/minute/kg. Hemoperfusion during carotid infusion increased Cl_{tb} to 29–152 ml/minute/kg. By increasing the rate of collection of jugular blood for extracorporeal hemoperfusion, Cl_{tb} was increased from 29 ml/minute/kg at 30 ml/minute to 83–152 ml/minute/kg at 60 ml/minute. The brain-to-body exposure ratio increased correspondingly from 18 (30 ml/minute) to 40–69 (60 ml/minute).

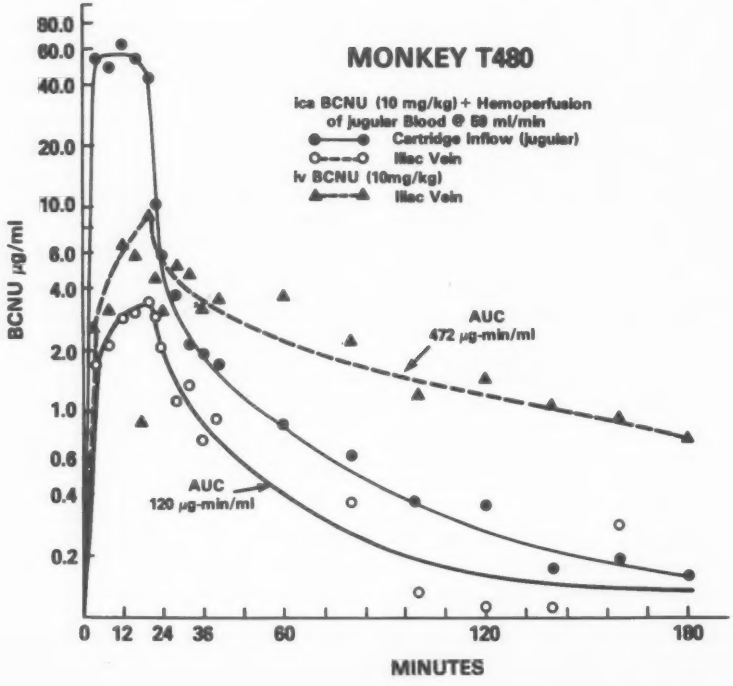
Our calculations indicate that most of the ica blood flow receiving BCNU v ıs shunted through the extracorporeal circuit (table 2). Of the estimated 20 ml/minute carotid blood flow into which BCNU was infused, 12.7–16.8 ml/minute was captured by the jugular catheter. The greatest fraction, 0.84, was captured at the highest pump rate, 60 ml/minute. The percentage of the administered dose which was removed by the cartridge was 72%–88%. These values were calculated from a material balance on the column inlet in which we assumed that the BCNU concentration in blood was the same as the measured concentration in plasma. The fraction of the dose removed by the cartridge results can be independently checked by comparing the systemic AUC values from table 1 with and without hemoperfusion. This calculation is shown in the last line in table 2. The two methods of calculation are in good agreement at the higher flow rates, but there is a significant unresolved discrepancy (0.46 vs 0.72) at the lower flow rate (table 2). It is, therefore, quite possible that the use of blood flow and plasma concentration overestimated f and the f-dependent calculations at the 30-ml/minute flow. Peak concentrations of jugular plasma (cartridge inflow) were 64–135 μg/ml; peak concentrations from the cartridge outflow never exceeded 0.26 μg/ml (fig 3). A comparison of the fraction of the dose of BCNU removed by the cartridge and the reduction in systemic exposure by hemoperfusion indicates that there was no quantitatively significant elimination of BCNU by the brain. This observation agrees with Levin et al (14), who compared the pharmacokinetics of BCNU following iv and carotid artery infusions in the squirrel monkey.

Myelosuppression was reduced by combining ica infusion with hemoperfusion. Both platelet and wbc counts dropped to lower levels during the 6 weeks following iv administration of BCNU (table 3). Figure 4 shows the weekly platelet and wbc counts of Monkey T480 for 6 weeks following each of the two treatments. It is noteworthy that the iv infusion followed the combined treatment in each animal. Therefore, the increased myelosup-

TABLE 2.—Calculated kinetics of hemoperfusion circuit

	Units	Monkey No.—		
		1172	T480	7614
Cartridge flow	ml/min	30	59	60
Carotid flow (Q)	ml/min	20	20	20
Fraction of carotid flow passing through cartridge (f)		0.64	0.66	0.84
Flow through cartridge contributed by carotid artery	ml/min	12.7	13.1	16.8
Flow through cartridge from systemic circulation (Q_s)	ml/min	17.3	45.9	43.2
Fraction of dose removed by cartridge		0.72	0.73	0.88
Reduction in systemic exposure by hemoperfusion		0.46	0.75	0.84


MONKEY 1172
ica BCNU (10mg/kg) + Hemoperfusion
of Jugular Blood @ 30ml/min
Cartridge Inflow (jugular)
Iliac Vein
iv BCNU (10mg/kg)
Iliac Vein
AUC
648 µg-min/ml
AUC
347 µg-min/ml
BCNU µg/ml
100.0
60.0
40.0
20.0
10.0
6.0
4.0
2.0
1.0
0.6
0.4
0.2
12
24
36
60
120
180
MINUTES


MONKEY T480
ica BCNU (10 mg/kg) + Hemoperfusion
of jugular Blood @ 59 ml/min
Cartridge Inflow (jugular)
Iliac Vein
iv BCNU (10mg/kg)
Iliac Vein
AUC
472 µg-min/ml
AUC
120 µg-min/ml
BCNU µg/ml
80.0
60.0
40.0
20.0
10.0
6.0
4.0
2.0
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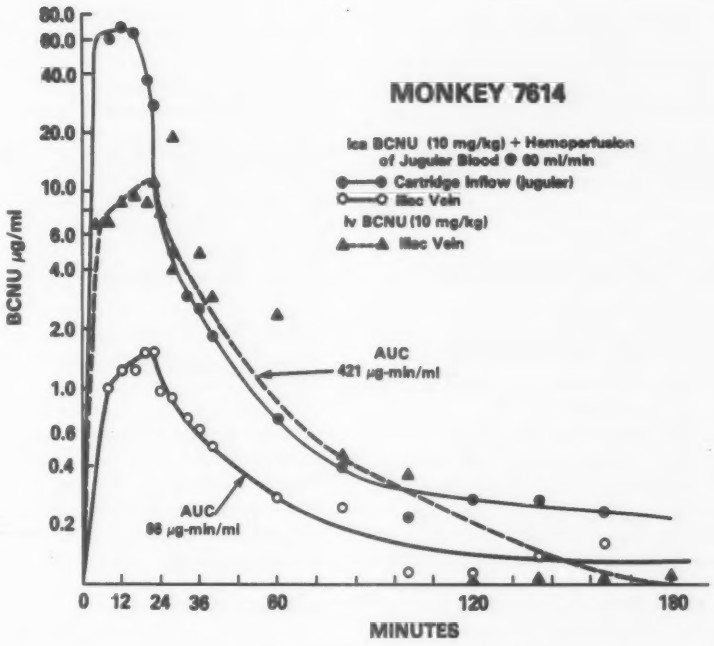

FIGURE 3.—Plasma BCNU levels during the 2 treatments in 3 rhesus monkeys. Plasma BCNU concentrations in blood from jugular vein (●——●) and iliac vein (O——O) after beginning intracarotid BCNU administration while hemoperfusing jugular blood at rates indicated. 6–9 wks later the animals received the same dose of BCNU iv and blood from the iliac vein was sampled for BCNU levels (Δ---Δ). Systemic exposure, the AUC, was reduced 46%–84% by the combined treatment.

TABLE 3.—Platelet and wbc count nadirs for 3 rhesus monkeys during 6-wk period following intracarotid BCNU combined with hemoperfusion of jugular blood or iv BCNU

Monkey No.	Lowest count × 10^3 cells/mm^3 following BCNU	
	Ica BCNU + jugular hemoperfusion	Iv BCNU
	Platelets	
1172	309	183
T480	196	85
7614	181	217
Mean	229	162
	Wbcs	
1172	6.8	4.2
T480	5.1	1.9
7614	5.4	2.8
Mean	5.8	3.0*

*$P < 0.01$.

pression following the iv treatments may have been at least partially due to greater cumulative BCNU doses.

DISCUSSION

The low therapeutic index of most anticancer therapies has stimulated interest in the development of methods for regional tumor treatment. These methods increase tumor exposure while maintaining tolerable systemic toxicity. Local irradiation, radioactive implants, chemotherapy by intrathecal or ip administration, and drug delivery by arterial infusion or isolation-perfusion tech-

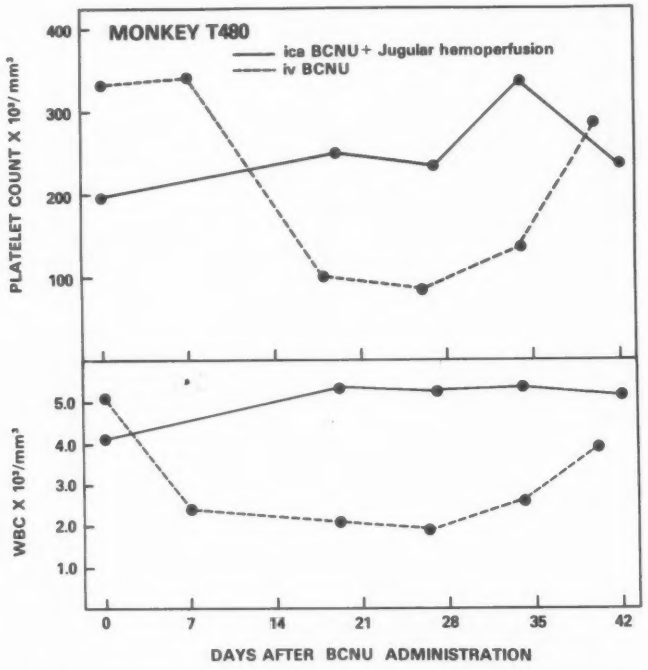

FIGURE 4.—Comparison of weekly platelet and wbc counts of 1 animal after receiving 10 mg/kg of BCNU by intracarotid administration during hemoperfusion of jugular venous drainage *or* iv. Iv treatment was given 9 wks following the treatment combining intracarotid BCNU with hemoperfusion.

niques exploit this principle. The last techniques, regional chemotherapy by arterial delivery and combining arterial delivery with partial vascular isolation of the tumor-bearing region, have been most frequently employed for tumors of the extremities and the head. Clinical application of these techniques for brain tumors began in the 1950s and 1960s (15-17), but lacked clinical effectiveness, in part because of contemporary technical limitations and ineffective anticancer drugs. Furthermore, it was then unrecognized that the total-body clearance of the drug being used was important in bestowing an advantage to arterial delivery.

Subsequently, pharmacokinetic analyses of intra-arterial drug administration established that the increased exposure of the perfused area is a function only of the ratio of the drug's Cl_{tb} to the blood flow of the perfused region (1-3,18). Simply stated, the advantages of intra-arterial infusion over iv infusion are that (*a*) greater drug concentrations perfuse the tumor if lower regional blood flow dilutes the drug less, and (*b*) since the drug is diluted by the general circulation after the first pass through the tumor and, on subsequent passes, perfuses the target area identically to an iv infusion, the fraction of the total exposure represented by the first passage through the tumor is greater if the drug is rapidly eliminated from the body. For a specific artery the kinetic advantage can be increased by selection of a drug with a high Cl_{tb} or by the application of techniques which increase Cl_{tb}. Accordingly, hemodialysis or hemoperfusion of blood from the general circulation could increase the pharmacokinetic advantage of regional arterial delivery. Since after arterial infusion venous drug concentration is greatest as it leaves the perfused organ, drug removal from the blood draining the perfused area is more efficient. This principle has been previously employed by others (19,20), although advances in technology indicate reassessment. Dedrick et al (13) have discussed the pharmacokinetics of intra-arterial delivery combined with extracorporeal hemoperfusion in detail.

Since one of the objects of our experiments was to perform a preclinical evaluation of this therapy for potential application to humans with malignant glial tumors, we simulated human conditions where possible. In the in vitro experiment we infused 400 mg of BCNU, about 240 mg/m^2 in a 70-kg human, into heparinized sheep whole blood which was being pumped through the hemoperfusion cartridge at 300 ml/minute, the flow rate of the human ica (21). After demonstrating quantitative drug removal at that rate of drug delivery and blood flow we performed a preclinical study in rhesus monkeys.

To reduce dilution of BCNU by blood from the collateral venous channels of the face, scalp, and neck entering the jugular vein in the neck, we positioned the tip of the catheter in the jugular bulb. In the first animal the blood from the jugular bulb was pumped at an estimated rate of unilateral jugular blood flow (30 ml/minute). Because the blood within a transverse venous sinus is a mixture of blood from both cerebral hemispheres, we pumped blood from the jugular bulb at twice the initial rate in the two remaining animals. By increasing the withdrawal of blood from the jugular bulb to a rate greater than the normal flow, we sought to create a sump effect which would reverse the direction of flow in the contralateral transverse sinus and allow collection of most of the blood draining the cerebral hemispheres.

The pharmacokinetic advantage and reduced systemic toxicity obtained by combining ica BCNU infusion with hemoperfusion of the jugular drainage justify consideration of its potential use in the treatment of malignant brain tumors. Requisite conditions for intra-arterial chemotherapy include localized tumor, tumor sensitivity to the drug being infused, and an anatomically consistent and accessible blood supply. These criteria are met by the most common primary brain tumor, glioblastoma multiforme, and BCNU.

Glioblastomas are local neoplasms; they rarely metastasize but invariably kill the patient by local growth. Following a brief clinical remission of weeks to months after surgical excision and radiation therapy, glioblastomas recur; 90% of these recurrences arise within the margin of the original tumor or within 2 cm of this margin as seen by computed tomographic scanning (22).

The nitrosoureas, BCNU and CCNU, have been shown to have activity in the treatment of malignant astrocytomas (4,23). The 36-week average survival following surgical excision and radiation therapy alone is increased to 52 weeks by the addition of BCNU at a dose of 80 mg/m^2 iv daily for 3 successive days every 6 weeks (4). The growth fraction of glioblastomas is < 0.35 (23,24). This precludes significant influence by a brief infusion of cell cycle-specific agents, but the antineoplastic activity of BCNU is not restricted to a particular phase of the cell cycle (23).

In vitro studies from two laboratories demonstrate decreased glioma cell survival with increasing exposure to BCNU. Kornblith et al (25), using a cytotoxicity assay, found a reciprocal relationship of BCNU concentrations with glioma cell survival and correlated the in vitro response with the in situ response in 11 of 14 patients. After an incubation period of 1 hour with an initial BCNU concentration of 6.6 μg/ml, there was no response of $> 50\%$ cytotoxicity to malignant astrocytomas cultured from 52 patients; 39 of 51 had $> 50\%$ cytotoxicity after a similar incubation period with initial BCNU concentrations of 13 μg/ml. When the initial concentration was increased to 33 μg/ml, at least 99% of the tumor cells were killed in 33 of 51 tumors. Although direct comparisons cannot be made with this system and in vivo BCNU concentrations due to differences in plasma and culture media, at initial BCNU concentrations of 6.6 and 33 μg/ml, and at 1-hour incubation, the estimated exposures to BCNU are 477 and 2385 μg-minute/ml, respectively

(13). Rosenblum et al (26), using a clonogenic assay, found less consistent decreases in in vitro survival with increasing BCNU concentrations, although in clinically responsive patients a dramatic response of the cell cultures was seen with 10-25 μg/ml of BCNU. For the 2 hours of incubation in this assay, the calculated exposures are 1028-2570 μg-minute/ml (13).

The in vitro responses suggest that in some patients sterilization of the tumor in situ might occur if tumor exposures > 2500-3000 μg-minute/ml could be safely achieved. Assuming human BCNU Cl_{tb} of 15 ml/minute/kg, a dose of 240 mg/m^2 into the ica of an adult (estimated ica flow, 300 ml/minute) of 1.7-m^2 body surface area yields a brain exposure of about 1800 μg-minute/ml. This is fourfold greater than the calculated exposure after administering the same dose iv and in accordance with the observations of Levin et al (14), who noted a fourfold to fivefold higher brain radioactivity in the middle cerebral artery distribution following carotid infusion than after iv infusion of ^{14}C-BCNU in the squirrel monkeys. Comparison of the calculated brain exposure achieved by ica infusion to that required for in vitro tumor sterilization suggests that if the dose of intracarotid BCNU could be increased by threefold to fivefold without brain or systemic toxicity, improved patient response might ensue.

Although adequate data are only now becoming available for humans, toxicity in the rhesus monkeys suggests that the primate brain is relatively resistant to toxicity by intracarotid BCNU. Crafts et al (8) delivered weekly doses as high as 20 mg/kg into the ica. At doses of 10 mg/kg weekly for 4 weeks they witnessed no cerebral toxicity, although myelosuppression occurred. Toxicity causing death in monkeys receiving 15 or 20 mg/kg of intracarotid BCNU arose from hepatic and renal failure rather than brain injury. One of our three animals had hemiparesis after a single intracarotid injection of 10 mg/kg. This resolved spontaneously over the ensuing 3 weeks. Since the catheter was in the ica for 30-60 minutes and there was a tendency for the monkeys to become hypotensive during extracorporeal circulation, the neurological deficit may have been ischemic in origin. We cannot eliminate brain injury by BCNU, however.

The cumulative experience from several clinics now includes many patients who received multiple doses of BCNU (100-250 mg/m^2) into the ica. Ocular toxicity, including blindness, occasionally complicates therapy (27), but only one patient experiencing irreversible brain toxicity attributable to intracarotid BCNU has been reported (28). Pruitt et al (29) infused doses as high as 600 mg/m^2 without clinically apparent brain injury, but significant myelosuppression occurred.

BCNU-induced systemic toxicity includes reversible and irreversible marrow suppression (30,31), pulmonary fibrosis (32), and renal failure (33). Severe myelosuppression with thrombocytopenia (platelet count < 60,000/mm^3) or leukopenia (wbc count < 2500/mm^3) occurs in 8% of patients receiving a cumulative dose of > 1200 mg/m^2 (30). Severe, irreversible nephrotoxicity and progressive pulmonary fibrosis complicate cumulative BCNU exposure and have led authorities to recommend a maximum total dose of 1500 mg/m^2 (32-34). Thus, the available evidence suggests that systemic rather than neural toxicity limits the tolerable dose of intracarotid BCNU and that higher doses may be safely administered if techniques reducing systemic exposure are successful.

Since intraspecies adult brain weight varies little with body size, brain exposure after carotid delivery would greatly vary between individuals with doses based on weight or body surface area. Similar problems exist in attempting interspecies comparisons. However, the ratio of brain mass to body mass of an average-size adult human and an average-size adult rhesus monkey approaches unity (13). We studied very large rhesus monkeys. The dose to the brain of 10 mg/kg of body weight was about 1 mg/g based on whole-brain weight. Levin et al (14) obtained similar doses to the brain in their small monkeys given 20 mg/kg of body weight. If (*a*) human brain sensitivity to BCNU is similar to that in the rhesus monkey, (*b*) the volume of infused brain is proportional to brain weight, and (c) systemic toxicity is not a problem, the intracarotid dose of BCNU might be safely increased to about 1.4 g. This is 20 mg/kg of body weight (740 mg/m^2) for a 70-kg patient or about three times the current dose. Such a threefold increase in dose exposes the tissue perfused only by the ica to an estimated 5000 μg-minute/ml. This significantly exceeds the calculated exposure required for a substantial response including sterilization of most tumors in the in vitro systems discussed previously. If extracorporeal hemoperfusion of jugular blood in the human replicates our experience with rhesus monkeys, then it should be possible to limit systemic toxicity at a dose of 1.4 g delivered into the ica.

Finally, in this communication we considered only the application of hemoperfusion of jugular drainage during intracarotid infusion of chemotherapy. The same pharmacokinetic principles apply to tumors and other diseases affecting other areas of the body in which arterial drug delivery and collection of regional venous drainage for drug removal can be performed.

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Inability of Dimethyl Sulfoxide To Increase Brain Uptake of Water-Soluble Compounds: Implications to Chemotherapy for Brain Tumors[1,2]

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There is conflicting evidence as to whether dimethyl sulfoxide (DMSO) can reversibly open the blood-brain barrier and augment brain uptake of water-soluble compounds, including anticancer agents. To investigate this, ^{125}I-human serum albumin, horseradish peroxidase, or the anticancer drug melphalan was administered iv to rats or mice, either alone or in combination with DMSO. Some animals received an additional ip priming dose of DMSO. The regional brain concentrations of each substance were measured after the animals were killed. DMSO administration did not significantly increase the brain uptake of any of the compounds as compared to control uptakes. These results do not support prior reports that DMSO increases the permeability of water-soluble agents across the blood-brain barrier. [Cancer Treat Rep 69:305–312, 1985]

A major limitation to the delivery of water-soluble drugs to the brain is the blood-brain barrier (BBB) (1,2), which is located at the level of the tight junctions between cerebrovascular endothelial cells (3). Whereas ultrastructural defects have been noted in the capillary endothelium of a number of malignant brain tumors (4,5), there is evidence that the BBB is partially intact in many tumors (6,7), particularly at the marginal edge of the invading cells (7,8), and completely intact in some tumors (9). Low blood-to-tissue transport of water-soluble compounds has been demonstrated in both experimental and human brain tumors (6,7). Limited BBB penetration during chemotherapy can lead not only to a poor initial therapeutic response but also to the induction of drug resistance.

Any technique that increases blood-to-tissue drug delivery would be advantageous in the treatment of brain tumors. Two interesting and proven techniques are osmotic BBB opening (10,11) and pentylenetetrazol BBB opening (12,13). Both can increase the brain delivery of anticancer agents that are normally excluded from the brain. Indeed, the osmotic method has been used clinically with some success (9,14,15).

Recently, there have been a number of reports that dimethyl sulfoxide (DMSO) can reversibly modify the BBB, and thereby increase brain uptake of intravascular anticancer drugs and the enzyme horseradish peroxidase (HRP) (16–18) and enhance a drug's anticancer activity (19–21). Since DMSO is a stable and relatively nontoxic solvent in which drugs can be dissolved, its effective use would represent an important contribution to brain tumor therapy. The proposal that DMSO can act as a carrier at the BBB for compounds with normally restricted cerebrovascular permeability is not new. It has been controversial for the last decade (22–27), but recently, opinion has swung to the affirmative view. Broadwell et al (16) reported that 0.25–0.5 ml of 2%–30% DMSO in saline, given iv to mice, reversibly opened the BBB to intravascular HRP. DMSO concentrations > 10% were most effective. Broadwell et al (17) later reported that BBB opening by iv DMSO in mice required a prior ip injection of DMSO. Best results were achieved with 0.5 ml ip of 10%–15% DMSO followed 5–30 minutes later by 0.25 ml iv of 10%–15% DMSO. Clinical trials were initiated on the basis of these reports (28–31).

On the other hand, Neuwelt et al (32) measured the cerebrovascular permeability of methotrexate, Evans blue-albumin, and hexosaminidase A, alone and in combination with DMSO, following administration of up to 90% DMSO iv and 30% DMSO intracarotidly to rats, but could not demonstrate alterations in cerebrovascular permeability to any of these tracers. They did not, however, use a prior ip injection of DMSO. Thus, the question remains open as to whether DMSO, if administered as de-

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scribed in the later publication of Broadwell et al (17), can increase the brain delivery of agents that are normally restricted by the BBB.

To examine the effects of DMSO on the BBB, we used ^{125}I-human serum albumin (HSA), HRP, and the anticancer drug melphalan as markers of BBB integrity, in combination with varying concentrations of DMSO, with or without a prior ip injection, and examined cerebrovascular permeability to these tracers. An abstract of this work has been published (33).

METHODS

Assessment of DMSO-Related BBB Opening Using ^{125}I-HSA and HRP

^{125}I-Human serum albumin.—^{125}I-HSA (Radiochemical Center, Amersham, Great Britain) was dissolved in either 25% or 50% DMSO (vol/vol in isotonic saline, 0.9% [wt/vol] NaCl), 99.9% pure by gas-liquid chromatography (Sigma Chemical Co, St. Louis, MO). This was injected iv (100 μCi/kg of body weight, 4 ml/kg) into four adult male Sprague-Dawley rats per group; the rats weighed 200–250 g. Six control animals received ^{125}I-HSA in isotonic saline. Thirty minutes later, each rat was anesthetized with chloroform. A midline thoracotomy was performed, the pericardium cut, and 2 ml of blood withdrawn from the left ventricle. The vasculature was cleared by infusing isotonic saline for 2 minutes at a pressure of 120 mm Hg through an aortic cannula. The blood sample was centrifuged (7000 × *g* for 1 minute), the plasma was removed, and it and the brain were stored on dry ice. Later, the brain was thawed and dissected into seven anatomically distinct regions which were placed into Beckman biovials, reweighed, and, with the aliquot of plasma, assayed for ^{125}I activity (Gamma 4000, Beckman Instruments, Palo Alto, CA).

Horseradish peroxidase.—HRP (30 mg/kg, Type II, Sigma) was dissolved in either saline or 25% or 100% DMSO and injected iv (4 ml/kg) into two awake adult male Sprague-Dawley rats per group. At 30 minutes the animals were anesthetized with chloroform and their cerebral vasculature was perfused with isotonic saline (120 mm Hg for 2 minutes). The brain was removed and placed on dry ice. Three coronal serial sections (20 μm thick) were cut by cryostat at approximately 300 μm intervals from the level of the caudate nucleus to the cerebellum. The first and third sections of each series were incubated by the Hanker technique (34) for the histochemical demonstration of HRP. Sections from both control and DMSO-treated animals were incubated together. Substrate controls from similar brain areas, in which H_2O_2 had been omitted, were also incubated for each animal. The second section in each series was stained with cresyl fast blue. The ultraviolet absorptions of both the HRP and HRP-reaction product were measured by spectrophotometry (Pye Unicam, Cambridge, Great Britain). The HRP-reaction product concentration within the sections was then quantified, at 490-nm wavelength, by scanning and integrating optical microdensitometry (Vickers M85 Optical Microdensitometer, York, Great Britain), identifying the brain areas from the stained sections.

Assessment of DMSO-Related BBB Opening Using HRP and Melphalan With Additional Prior Ip Injection of DMSO

Horseradish peroxidase.—HRP (100 mg/kg, Type VI, Sigma) was injected iv into eight anesthetized adult male Sprague-Dawley rats (sodium pentobarbital, 40 mg/kg ip). Three rats were injected ip with 2.0–2.5 ml of isotonic saline followed 15 minutes later by iv HRP in saline (4 ml/kg). The remaining rats were injected ip with 2.0–2.5 ml of 15% DMSO followed 15 minutes later by iv HRP in either 15% DMSO (4 ml/kg, three animals) or 30% DMSO (4 ml/kg, two animals). All animals were killed 30 minutes following HRP administration. The tissue was prepared and stained and the HRP-reaction product quantified as previously described.

Melphalan.—Nine adult male Fisher 344 rats, weighing 200–250 g, were anesthetized (sodium pentobarbital, 40 mg/kg ip). Catheters filled with sodium heparin (100 IU/ml in isotonic saline) were tied into a femoral artery and vein. Three animals were injected ip with 2.0–2.5 ml of 15% DMSO followed 15 minutes later by iv melphalan (10 mg/kg, Burroughs Wellcome, Research Triangle, NC) in 15% DMSO (4 ml/kg). Six animals were injected ip with 2.0–2.5 ml of isotonic saline followed 15 minutes later by iv melphalan alone (10 mg/kg, 4 ml/kg). Arterial blood samples were removed at zero time and from 15 seconds to 30 minutes were centrifuged at 7000 × *g* for 1 minute; the plasma was removed and stored on dry ice. At 30 minutes, cerebrospinal fluid (CSF) was collected by percutaneous sampling from the cisterna magna (35). Both the CSF and brain were stored on dry ice.

An additional 30 rats were anesthetized (sodium pentobarbital, 40 mg/kg ip). One-half of the rats were injected iv (4 ml/kg) with melphalan alone (10 mg/kg) following a 15-minute prior ip injection of 2.0–2.5 ml of isotonic saline. The remainder were injected iv (4 ml/kg) with melphalan (10 mg/kg) in 15% DMSO following a 15-minute prior ip injection of 2.0–2.5 ml of 15% DMSO. At intervals from 15 minutes to 4 hours following melphalan administration, blood, CSF, and brain were taken from both control and DMSO-treated animals using at least three animals per time point. The blood was centrifuged (7000 × *g* for 1 minute) and the plasma, CSF, and brain were stored on dry ice. All brains were later thawed and the cerebellum and cerebral cortex were removed, weighed, and, as were the plasma and CSF samples, analyzed for melphalan by high-pressure liquid chromatography (36).

Calculations

The brain uptake of ^{125}I-HSA was calculated from the ratio of the concentration of tracer in the brain (C_{Br}) to that in the plasma (C_{Pl}) at 30 minutes. Because the cerebral vasculature was cleared with saline at the time of death, C_{Br} represents the tracer in the brain parenchyma alone, and a correction for blood volume is not required. It is probable, however, that not all the blood was washed out and that some small fraction, approximately 5%, was retained. This would be the same in controls and DMSO-treated animals and therefore can be disregarded. Thus, C_{Br}/C_{Pl} approximates the actual brain space at 30 minutes.

The brain uptake of melphalan was evaluated as follows. Rats were killed 30 minutes following iv melphalan administration. During the 30 minutes the arterial drug concentration markedly exceeded the brain concentration; back diffusion of drug from brain to plasma therefore was negligible. Thus, the data can be analyzed according to the two-compartmental model of Ohno et al (10) for plasma-to-brain entry, where brain uptake is given as

$$dC_{Br}/dt = PA.f.C_{Pl}. \quad [1]$$

In equation 1, P (cm/seconds) = the cerebrovascular permeability; A (cm^2/g of brain or cm^{-1}) = the capillary surface area; t = time in seconds; and f = the unbound fraction of drug in the plasma [which was determined by ultracentrifugation (37)]. C_{Br} (μg/g), the brain parenchymal drug concentration, was calculated from the net regional brain concentration by subtracting the intravascular drug concentration at the time of death (T). The intravascular concentration equaled the whole blood concentration (μg/ml) at time T, multiplied by the regional blood volume (ml/g of brain). The regional blood volume equals the ^{125}I-HSA space of unperfused brains at 2 minutes. For the cerebral cortex and cerebellum, the regional volumes were 1.7% and 2.4%, respectively.

Equation 1 can be integrated to the time of death (T) to give PA in terms of the arterial plasma concentration integral.

$$PA = \frac{C_{Br}\ (T)}{f \int_0^T C_{Pl}\ dt} \quad [2]$$

For melphalan, f was found to be equal to 0.4 in the plasma concentration range measured up to time T.

Statistical analysis to assess differences between controls and DMSO-treated animals was undertaken using the Student's t test. In cases of multiple comparisons to the controls (tables 1-4), Dunnett's t test was also employed (38). Significance was $P < 0.05$.

RESULTS

Table 1 presents the mean regional brain space of ^{125}I-HSA at 0.5 hour in animals injected iv with ^{125}I-HSA in either saline (controls), 25% DMSO, or 50% DMSO. There was no significant difference in the ^{125}I-HSA space between the controls and animals injected with either dose of DMSO in any of the seven brain areas studied.

Table 2 presents mean integrated optical densities of HRP-reaction product, measured at 490-nm wavelength, in brains of animals administered HRP in either saline (controls) or 25% or 100% DMSO. There was no significant difference between means in controls and at either dose of DMSO in any of the six brain areas examined. The iv administration of 100% DMSO did, however, cause some central nervous system toxicity. One animal developed seizures, died within 10 minutes, and was not included in the study. Of the two animals included, one developed hind leg twitching. All animals administered HRP in DMSO developed hematuria.

Further experiments were undertaken to examine the effects of an additional ip priming dose of DMSO on brain uptakes of HRP and melphalan which were administered iv in DMSO. Table 3 presents mean integrated optical densities of HRP-reaction product in various brain regions, following iv HRP in saline, in 15% DMSO, or in 30% DMSO after an ip priming dose of 2.5 ml of

TABLE 1.—Effects of 25% and 50% DMSO on the 0.5-hr uptake of ^{125}I-HSA

Brain area	Experimental protocol	^{125}I-HSA space × 10^{-3}*
Cerebral cortex	Control	0.46 ± 0.05
	25% DMSO	0.50 ± 0.06
	50% DMSO	0.40 ± 0.05
Caudate nucleus	Control	0.27 ± 0.03
	25% DMSO	0.36 ± 0.08
	50% DMSO	0.36 ± 0.06
Hippocampus	Control	0.39 ± 0.02
	25% DMSO	0.42 ± 0.06
	50% DMSO	0.34 ± 0.04
Thalamus	Control	0.32 ± 0.08
	25% DMSO	0.39 ± 0.07
	50% DMSO	0.42 ± 0.05
Hypothalamus	Control	1.02 ± 0.04
	25% DMSO	0.94 ± 0.10
	50% DMSO	1.25 ± 0.10
Pontine area	Control	0.52 ± 0.05
	25% DMSO	0.59 ± 0.06
	50% DMSO	0.47 ± 0.06
Cerebellum	Control	0.55 ± 0.09
	25% DMSO	0.47 ± 0.06
	50% DMSO	0.60 ± 0.06

* Mean ± SEM (6 control animals, 4 animals/DMSO group). No significant difference was found between control mean and means of DMSO-treated animals ($P < 0.05$): ^{125}I-HSA space = $\frac{\text{cpm/mg of brain}}{\text{cpm/}\mu\text{l of plasma}}$.

TABLE 2.—Effects of 25% and 100% DMSO on the 0.5-hr brain uptake of HRP

Brain area	Experimental protocol	No. of areas scanned (500 μm^2)	Integrated optical density × 10^{-2}*
Cerebral cortex	Control	15	16.3 ± 0.33
	25% DMSO	13	17.4 ± 0.72
	100% DMSO	14	16.8 ± 0.38
Caudate nucleus	Control	15	18.2 ± 0.54
	25% DMSO	12	17.0 ± 0.94
	100% DMSO	12	17.2 ± 0.68
Hippocampus	Control	15	18.0 ± 0.79
	25% DMSO	10	19.4 ± 1.06
	100% DMSO	14	17.3 ± 0.63
Thalamus	Control	15	20.6 ± 1.11
	25% DMSO	12	20.9 ± 0.98
	100% DMSO	13	21.7 ± 0.82
Pontine area	Control	14	19.4 ± 0.81
	25% DMSO	10	20.3 ± 0.81
	100% DMSO	15	20.9 ± 0.53
Cerebellum	Control	15	25.4± 1.61
	25% DMSO	15	22.6 ± 1.23
	100% DMSO	15	23.9 ± 1.47

* Mean ± SEM (2 animals/group). Integrated optical density was measured at 490 nm. No DMSO mean differed significantly from the control mean at each region ($P > 0.05$).

15% DMSO. With the exception of the caudate nucleus in animals administered 15% DMSO and of the corpus callosum in those administered 30% DMSO, there was no significant difference between control and experimental means in any of the seven brain areas studied. In animals given 15% DMSO, the integrated optical density of HRP-

TABLE 3.—Effects of 15% and 30% iv DMSO + prior ip injection of DMSO on the 0.5-hr brain uptake of HRP

Brain area	Experimental protocol	No. of areas scanned (500 μm^2)	Integrated optical density × 10^{-2}*
Cerebral cortex	Control	46	10.2± 0.33
	15% DMSO	47	9.4 ± 0.29
	30% DMSO	40	10.8 ± 0.28
Caudate nucleus	Control	20	12.3 ± 0.35
	15% DMSO	21	10.9 ± 0.55†
	30% DMSO	20	13.2 ± 0.32
Hippocampus	Control	20	10.4 ± 0.22
	15% DMSO	20	10.2 ± 0.42
	30% DMSO	20	9.9 ± 0.21
Thalamus	Control	20	11.5 ± 0.22
	15% DMSO	20	10.6 ± 0.39
	30% DMSO	21	11.6 ± 0.21
Corpus callosum	Control	20	8.0 ± 0.31
	15% DMSO	20	8.3 ± 0.30
	30% DMSO	18	9.1 ± 0.34†
Pontine area	Control	21	9.5 ± 0.18
	15% DMSO	20	9.0 ± 0.25
	30% DMSO	20	9.9 ± 0.21
Cerebellum	Control	21	10.7 ± 0.38
	15% DMSO	21	10.0 ± 0.42
	30% DMSO	20	10.2 ± 0.19

* Mean ± SEM (3 animals/control and 15% DMSO groups; 2 animals/30% DMSO group). Integrated optical density was measured at 490 nm.

† Differs significantly from control mean ($P < 0.05$) (Dunnett's t test).

TABLE 4.—Serum osmolality 2 mins following administration of DMSO to mice

Experimental conditions	Osmolality, mosmols*
Control, 0.25 ml of saline iv	306 ± 1.6 (8)
15% DMSO ip (0.5 ml) + 0.25 ml of saline iv	350 ± 2.4† (6)
15% DMSO ip (0.5 ml) + 0.25 ml of 15% DMSO iv	430 ± 4.9† (6)

* Mean ± SEM (No. of animals in parentheses).
† Differs significantly from controls ($P < 0.05$) (Dunnett's t test).

reaction product was significantly less than in the controls (88.6%), while in those given 30% DMSO, it was significantly greater (113.8% of the controls). These differences are minimal and not much more than expected by chance.

Table 5 presents mean calculated PAs (equation 2) for melphalan in the cerebral cortex, cerebellum, and CSF of animals administered the drug alone (controls) or in DMSO. There was no significant difference between the controls and DMSO-treated animals. Figures 1 and 2 present plasma and brain melphalan concentrations from 15 minutes to 4 hours after melphalan was administered alone or in DMSO. There was also no significant difference between plasma concentrations of control and DMSO-treated animals at any time point. The areas under the concentration-time curves (AUC, determined by the trapezoidal rule) were 21.6 and 21.9 hr·μg/ml, respectively, for melphalan alone and in DMSO. Furthermore, there was no significant difference in melphalan concentration between control and DMSO-treated animals in either the cerebral cortex or cerebellum at any time point (fig 2). The cerebral cortex AUC was 2.5 and 2.4 hr·μg/g for the melphalan alone and for melphalan in DMSO, respectively; AUCs for the cerebellum were 5.1 and 4.9 hr·μg/g, respectively.

DISCUSSION

These experiments provide no evidence that DMSO enhances the penetration of water-soluble agents across the BBB, whether or not a priming dose has been given. Three compounds of varying molecular weight were studied (melphalan, 305 daltons; HRP, 40,000 daltons; and ^{125}I-HSA, 69,000 daltons). The brain uptake of each was quantified by a different technique, but the results were similar and did not indicate that DMSO increased their permeability at the BBB.

In a final attempt to reproduce the BBB opening described by Broadwell et al (16–18), their exact experiments were repeated in nine awake adult male white mice weighing 25–30 g. Three control mice received iv HRP in saline; the remainder received iv HRP in DMSO (three in 15% DMSO and three in 30% DMSO) following a 15-minute prior ip injection of 15% DMSO. The animals were killed at 30 minutes and the brains were prepared, cut, and stained as described. There was no increase in the brain penetration of HRP. We have no ready explanation for the staining reported by Broadwell et al (16–18).

DMSO can alter protein configuration (39,40) and enzyme activity (41); these effects might change membrane permeability, since the outer matrix of cell membranes is partially comprised of proteins. Such chemical alterations by DMSO, however, occur at higher concentrations than those used by Broadwell et al (16–18) and are usually irreversible (39,42). DMSO-induced changes in skin and bladder permeability have been related to its toxic action, resulting in histologically identifiable structural alterations (43,44). It is unlikely that such action would account for the innocuous, reversible BBB opening described by Broadwell et al (16–18). These investigators did, however, describe vesicles in the cerebral endothelium following DMSO, which also have been noted after a number of toxic insults to the BBB (1,2).

It remains possible that the findings of Broadwell et al (16–18) resulted from a DMSO-induced elevation of serum osmolality. We measured serum osmolality with a vapor pressure osmometer (Wescor Inc, Logan, UT) in mice 2 minutes following the iv administration of 15% DMSO (table 4); it was significantly increased. Despite this, however, the iv administration of 100% DMSO, which would have caused a similar or greater increase, produced no BBB opening in our study, and Neuwelt et al (32) reported that the intracarotid infusion of 30% DMSO caused only minimal BBB opening which, like the administration of 100% DMSO in our study, was accompanied by neurological toxicity.

The administration of a hypertonic load to experimental animals can shrink the brain and tear cerebral blood vessels, causing hemorrhages (S. I. Rapoport and T. S.

TABLE 5.—Effects of DMSO on cerebrovascular permeability product (PA) of melphalan

Experiment	Brain region PA ($s^{-1} \times 10^{-5}$)*		
	Cerebral cortex	Cerebellum	CSF
Melphalan alone (10 mg/kg iv)	6.2 ± 0.5	11.4 ± 1.0	8.8 ± 0.7
Melphalan (10 mg/kg) in 15% DMSO iv + 15% DMSO ip	8.3 ± 1.2	14.5 ± 1.0	7.3 ± 0.7

* Mean ± SEM (6 animals for melphalan alone, 3 for melphalan + DMSO). No DMSO mean differed significantly from the control mean ($P > 0.05$).

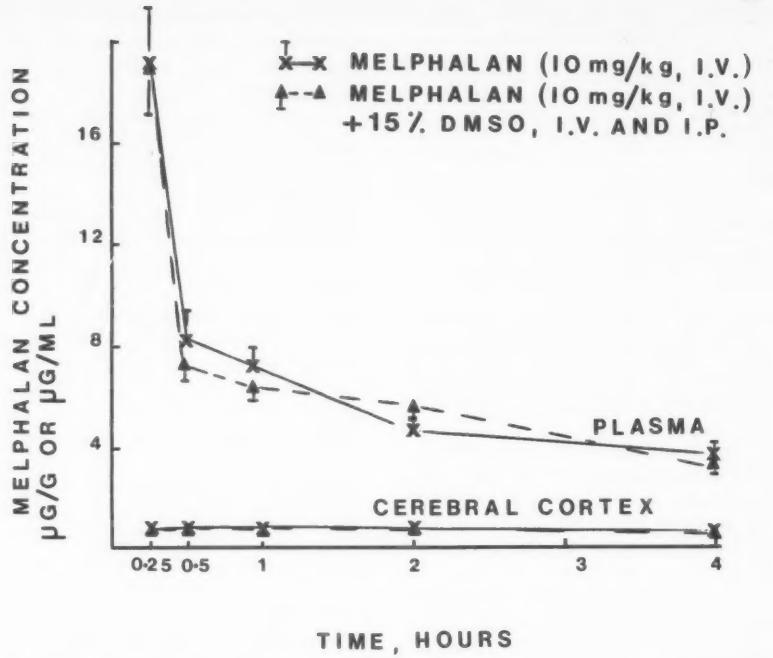

FIGURE 1.—Plasma and brain melphalan concentrations (± SEM) following iv administration of melphalan alone (10 mg/kg) or melphalan (10 mg/kg) + 15% DMSO with an additional prior ip injection of 15% DMSO in the rat.

Reese, unpublished observation). Dodge et al (45) reported that an increase in serum osmolality by ip sodium chloride administration to > 400 mosmols can open the BBB. A similar increase was induced by DMSO. However, unlike clinically used osmotic agents, DMSO has a high permeability at cell membranes and freely pene-

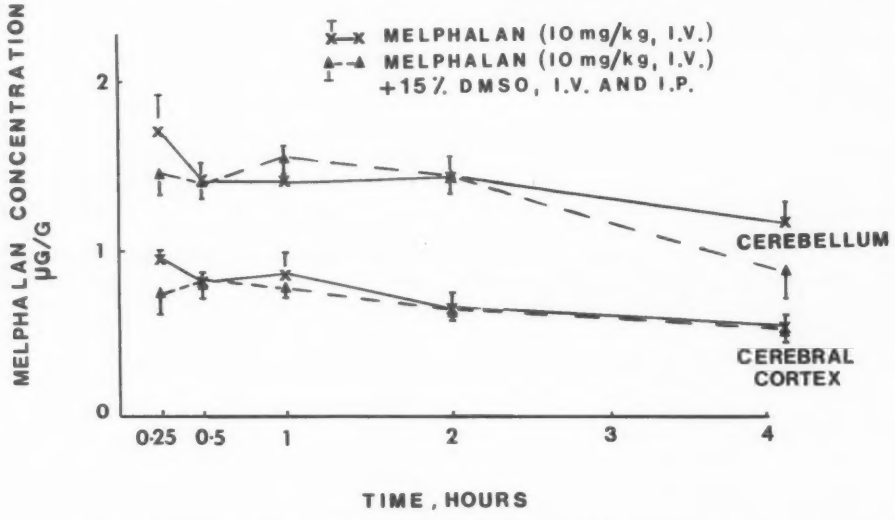

FIGURE 2.—Brain melphalan concentrations (± SEM) following iv administration of melphalan alone (10 mg/kg) or melphalan (10 mg/kg) + 15% DMSO with an additional prior injection of 15% DMSO in the rat.

trates the brain. Thus, it is highly unlikely that the DMSO-induced increase in serum osmolality would markedly shrink the brain or open the BBB (2,46).

It has been reported that DMSO, administered iv to humans, increases serum osmolality linearly with respect to its concentration over the range considered therapeutically relevant (0–10 ml/L) (47). Further, that due to its long plasma half-life of 4 days (48), the administration of large or consecutive doses can increase serum osmolality to levels incompatible with life. In conjunction with this, recent clinical trials have shown that DMSO will not potentiate the antitumor activity of cyclophosphamide against squamous cell carcinoma of the lung (29). In fact, DMSO may inhibit the alkylating capacity of chlorambucil and possibly of other similar agents (49). Finally, clinical trials have demonstrated that DMSO will not increase the brain penetration of either cyclophosphamide or doxorubicin in patients with brain tumors (30,31). Thus, while it would be useful to have an agent to enhance brain uptake of therapeutically relevant water-soluble drugs, DMSO will not fulfill this role. In view of this, our evidence suggests that further clinical trials attempting to use DMSO to increase the brain uptake of water-soluble drugs are not warranted. Further, since animal toxicity studies have shown that anticancer agents, in particular doxorubicin, are neurotoxic when given after barrier modification (50), toxicity studies should always precede the initiation of such clinical trials.

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Severe Central Nervous System Toxicity From High-Dose Cytarabine: Expressive Aphasia Occurring After the Second Day of Treatment[1]

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A patient with acute erythroleukemia secondary to polycythemia vera was treated iv with high-dose cytarabine at a dose of 3 g/m² every 12 hours. After the fourth dose, he developed an expressive (Broca's type) aphasia and somnolence, which progressed until after the treatment was stopped at the seventh dose. The somnolence cleared partially during the next 2 weeks but recurred after cytarabine was restarted at a dose of 100 mg/m² daily for 7 days. The patient died 5 days later of refractory extreme thrombocytopenia and bleeding. The normal spinal fluid obtained during life and the postmortem findings were consistent with previously reported findings with central nervous system toxicity from high-dose cytarabine. Three unusual aspects of this case are early onset of symptoms, expressive aphasia as the dominant manifestation, and exacerbation of symptoms with standard doses (100 mg/m²/day). [Cancer Treat Rep 69:313–314, 1985]

High-dose cytarabine (HDARA-C), usually given iv at a dose of 3 g/m² every 12 hours for 12 doses per course, has recently been found to be useful as induction therapy for patients with refractory acute nonlymphocytic leukemia and lymphoma (1–3).

In addition to such well-documented side effects of HDARA-C as nausea and vomiting, conjunctivitis, and prolonged pancytopenia, several investigators have recently noted the occurrence of cerebral and cerebellar dysfunction (4–7). In all but two reported cases, central nervous system (CNS) side effects did not occur before a cumulative cytarabine (ARA-C) dose of 24 g/m². We now report the occurrence of a Broca's type expressive aphasia which began after the second day of treatment when the cumulative dose was 12 g/m². The patient worsened until the drug was stopped on the fourth day, after 21 g/m² had been given.

CASE REPORT

In December 1982 a previously healthy, left-handed, 51-year-old white male was diagnosed as having polycythemia vera, according to the criteria of the Polycythemia Vera Study Group (8). He was treated only with phlebotomy to maintain a hematocrit of approximately 42%. There were no complications from this treatment.

In June 1983 the patient's family physician noted a steadily decreasing neutrophil count. Bone marrow examination in early July disclosed acute erythroleukemia. Because he was asymptomatic, he was not treated for this condition until 3 weeks later, when he was admitted to the hospital because of the sudden development of a spiking fever. Physical examination was normal except for a small vesicular lesion on the lower lip. Multiple cultures from all sites, including spinal fluid, were negative, though the patient remained febrile despite treatment with broad-spectrum antibiotics. The initial spinal fluid chemistries and cell count were normal. In early August he began chemotherapy with HDARA-C, 3 g/m² (7.2 g, total dose) iv every 12 hours and received a total of seven doses. Initially, the patient tolerated the chemotherapy well, except for nausea and vomiting during the 75-minute infusion. Prior to the fifth dose, the patient became lethargic and communicated verbally with great difficulty. He had long pauses between phrases and slurred speech. He exhibited no tremor, dysdiadochokinesis, past pointing, or change in legibility of signature. He could follow complex commands and identify objects but indicated by pounding the bed his frustration at being unable to express himself verbally. Neurologic evaluation was normal other than a slight bilateral (slightly greater on the left) pronator drift on arm extension. By the following day he was markedly obtunded. Lumbar puncture and computed tomography

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scan of the head were normal; EEG showed diffuse slowing. Because his symptoms were believed to be due to HDARA-C, this therapy was discontinued after the seventh dose (total dose, 21 g/m^2).

Other medications the patient was taking at the time he developed symptoms included the following: iv amikacin and piperacillin and oral nystatin as empiric therapy for the presumed infection; dexamethasone (10 mg) and prochlorperazine maleate (10 mg) iv prior to each dose of HDARA-C (dexamethasone given only for the first three doses) for prevention of nausea; dexamethasone 0.1% opthalmic drops, four times daily to help prevent conjunctivitis; and acetaminophen for fever.

Six days later the patient was much less somnolent, but he remained nearly completely aphasic. He was able only to say names, short phrases, and bursts of coprolalia. A bone marrow examination at this time showed persistent disease. He then received standard antileukemic therapy, including ARA-C at a dose of 100 mg/m^2 daily for 7 days by continuous infusion. Six days after the completion of the second course of chemotherapy, the patient's condition again declined. He became nearly completely unresponsive. Repeat lumbar puncture and computed tomography scan of the head were normal. EEG again showed diffuse slowing. The bone marrow at this time was aplastic. Five days later the patient, who had developed a lack of response to platelet transfusions, suddenly vomited a large amount of blood, became hypotensive, developed diffuse cutaneous purpura, and died.

Postmortem Findings

An autopsy, performed 2 hours after the patient's death, confirmed the clinical impression of exsanguination due to thrombocytopenia as the cause of death. All cultures of material obtained at postmortem examination were negative.

The brain showed no evidence of swelling, herniation, or shifts and no areas of infarction or intracerebral hemorrhage. In the cerebellum there was a disseminated, moderate loss of Purkinje cells in addition to the acute hypoxic changes seen throughout the brain. Bergmann's astrocytosis was seen in the region of loss of the Purkinje cells. There was no leukemic infiltration intracranially. The brain tissue was cultured for herpes simplex virus, and this was negative after 1 month.

DISCUSSION

The frequency of reported CNS side effects from HDARA-C has varied from 7.5% (6) to 28% (7). Most of the side effects were cerebellar, such as ataxia, dysarthria, ocular palsies, and intention tremor. Less commonly, cerebral dysfunction characterized by cognitive dysfunction, personality change, and somnolence have been reported (4–7). Toxicity appears to be related to cumulative dose (5–7). Only two reported patients have developed CNS toxicity at a cumulative dose < 24 g/m^2: one was at 3 g/m^2 (7) and the other was at 15 g/m^2 (4).

The reported pathology has been mostly Purkinje cell loss, predominantly in the depths of primary and secondary sulci, and Bergmann glial proliferation in the same areas (4,5,7).

We report the patient described here because of his unusual toxic reaction to HDARA-C. Most prominent was the early onset of a Broca's type aphasia which persisted throughout the last 4 weeks of his life. Most patients previously described with CNS toxicity had complete resolution or marked improvement in their symptoms. Our patient became more alert for a while, but then relapsed after further treatment with low-dose ARA-C. His aphasia, however, always remained severe.

One possible contributing factor to this unusual toxicity was the patient's large size (2.4-m^2 body surface area). Although he was muscular rather than obese, it is possible that his CNS was not proportionally as large as his body. This may have resulted in a dose of ARA-C which was greater per milliliter of spinal fluid or gram of brain tissue than in most other reported individuals.

Although the Purkinje cells in the cerebellum appear most sensitive to HDARA-C, what was seen pathologically did not explain all the clinical observations. In the few previously described cases, pathologic findings have been limited for the most part to the cerebellum, even when there was marked cerebral dysfunction as well (4,5,7).

This patient was judged to have CNS toxicity from HDARA-C because of the pathologic changes similar to those previously described and because there was no other explanation for the untoward clinical findings either during life or at postmortem examination.

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Coagulopathy Induced by Continuous Infusion of High Doses of Human Lymphoblastoid Interferon[1,2]

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Seven patients with myeloblastic leukemia were treated for 10 days with high-dose (15 or 30 million units/m^2/day), human lymphoblastoid interferon (Wellferon) by continuous iv infusion. All patients developed prolonged activated partial thromboplastin time, and four developed prolonged prothrombin time. Factor assays demonstrated low levels of II, VII, IX, X, and XII. Coagulation abnormalities improved after discontinuation of interferon therapy. [Cancer Treat Rep 69:315–317, 1985]

Human lymphoblastoid interferon (Wellferon) was used in a phase I–II clinical trial for children with refractory myelocytic leukemia, based on in vitro (1–3) and clinical reports of potential efficacy (4–6). A high-dose continuous iv infusion of interferon was used in an effort to improve the therapeutic efficacy (4–7). This report describes a significant coagulopathy which occurred in patients receiving this dosage schedule.

PATIENTS AND METHODS

Seven patients (1.75–19 years of age) with myelocytic leukemia in relapse and refractory to standard therapy were treated with constant-infusion human lymphoblastoid interferon. This trial was approved by the institution's Clinical Trials Committee and informed consent was obtained. Criteria for entry required a diagnosis of refractory myelocytic leukemia, recovery from previous therapy, and normal hepatic function.

The lymphoblastoid interferon (Wellferon) used in this trial was a mixture of human alpha interferon subtypes, (> 85% pure). It was produced by cell culture and supplied by Burroughs Wellcome Co (1). Activity is expressed in megaunits (MU): 1 MU equals 1×10^6 reference units. Therapy consisted of a 10-day continuous iv infusion at a dose of 30 MU/m^2/day in two patients, and 15 MU/m^2/day in five.

Daily coagulation assays for the five patients treated with 15 MU/m^2/day included prothrombin time (PT) (normal, 9.5–13 seconds), activated partial thromboplastin time (APTT) (normal, 23.5–40 seconds), and fibrinogen (normal, 125–445 mg/dl). Liver function tests were analyzed daily. Fibrin degradation products were measured when indicated (Wellcome Reagents, Beckenham, United Kingdom). Coagulation factors were measured from frozen plasma using a standard technique that measures the correction obtained when the patient's plasma is added to factor-deficient plasma. The normal range for all factors is 50%–150%.

RESULTS

The first two patients received 30 MU/m^2/day of interferon and both developed overt bleeding by Day 7, with platelet counts < 10,000/mm^3. Although bleeding was controlled by platelet transfusions, further evaluation revealed a prolonged APTT in both patients (60 and 50 seconds). The remaining five patients received 15 MU/m^2/day and did not develop bleeding; however, they all developed abnormal APTT and three developed abnormal PT (table 1). No patient developed consistently elevated fibrin degradation products or progressive depression of the fibrinogen. Coagulation abnormalities resolved a few days after discontinuation of the interferon

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TABLE 1.—Coagulation assays in patients receiving 15 MU/m²/day*

Patient	Assay†	Preinfusion	Day 1	2	3	4‡	5	6	7	8	9	10§	14
3	APTT	47.7	55.7	63.2	68	84	86.3	52	56			24	
	PT	11.6	11.6	14.1	13.5	12.4	11.8	11.4	13.5			13.7	
4¶	APTT	40	43	43	56.7	60.2	73.5	50	57	55.3	65.6	62.5	38
	PT	12.5	12.5	12.5	12.5	11.8	10.8	11.8	12.7	12.6	14	14.5	12.5
5¶	APTT	39.3	36	39	39.6	39.4	41.0	42.1	43	50	55	66	46
	PT	13	13.5	13.3	12.9	12.6	12.1	12.5	13.4	15.2	16.6	20	18
6¶	APTT	38	40.7	50.7	53.5	52	47	51	47	50	43.6	40	37.5
	PT	12.3	11.5	11.4	11.8	11.9	12.3	12.2	11.8	11.8	11	11.2	11.7
7¶	APTT	37	42	46.9	41	45.5	52.6	51.6	45.7	38.4	42.5	39	33
	PT	11.7	12.9	12.5	11	11	11.8	11	11.3	10.7	11.1	10.5	12

* Values = seconds.
†Normal APTT = 23.5–40 seconds; normal PT = 9.5–13 seconds.
‡Day interferon was discontinued in Patient 3.
§Day interferon was discontinued in Patients 4–7.
¶Vitamin K therapy given in Patients 4 and 5, Day 11 only; in Patient 6, Days 9–11; and in Patient 7, Days 1–11.

(table 1). Vitamin K appeared to improve, but did not completely correct or prevent, these abnormalities (table 1), suggesting that vitamin K-dependent and -independent factors were affected.

Coagulation factors, studied retrospectively in patients who received 15 MU/m²/day demonstrated low levels of factors II, VII, IX, X, and XII. Factors V and VIII were normal in all patients studied. Representative factor assays (Patients 3 and 4) are shown in table 2. Steady-state serum interferon levels for patients receiving 15 MU/m²/day ranged from 93 to 189 units and were not correlated with the coagulation abnormalities. No patient developed detectable hepatotoxicity.

DISCUSSION

A previously unrecognized coagulopathy developed in patients receiving high-dose continuous human lymphoblastoid interferon. This coagulopathy was usually not associated with overt bleeding. Interferon had a greater effect on the APTT than the PT, and depressed the activity of factors II, VII, IX, and X (vitamin K-dependent),

TABLE 2.—Coagulation factor assays*

Factor	Preinfusion	Day 2	4†	5	7	10‡	14§
		Patient 3					
VII (%)	74	ND	ND	—	35	—	—
IX (%)	57	39	22	—	47	—	—
X (%)	80	18	44	—	44	—	—
XII (%)	63	45	28	—	190	—	—
Fibrinogen (mg/dl)	238	155	140	—	205	—	—
Platelet count (x 10^3/mm³)	300	100	120	—	130	—	—
Fibrin degradation product¶	NEG	NEG	NEG	—	NEG	—	—
		Patient 4					
II (%)	ND	—	ND	45	—	25	45
VII (%)	81	—	70	75	—	52	111
IX (%)	70	—	41	64	—	25	40
X (%)	ND	—	79	65	—	24	62
XII (%)	67	—	81	62	—	56	64
V (%)	79	—	109	ND	—	ND	ND
VIII (%)	200	—	104	103	—	90	ND
Fibrinogen (mg/dl)	180	—	185	200	—	318	260
Platelet count (x 10^3/mm³)	141	—	66	73	—	36	40
Fibrin degradation product¶	1:10	—	1:10	1:20	—	1:10	1:20

* ND = not done; NEG = not detected.
†Day interferon was discontinued in Patient 3.
‡Day interferon was discontinued in Patient 4.
§3 days after vitamin K (phytonadione), 5 mg iv, in Patient 4.
¶Fibrin-related antigens measured by Thromo-Wellcotest; highest dilution with agglutination.

and factor XII (vitamin K-independent). The decreased factor XII activity might explain the greater sensitivity of the APTT and the failure of vitamin K to completely correct this coagulopathy.

Interferon appears to affect vitamin K because of the depressed vitamin K-dependent factors and an apparent response to vitamin K administration. Our patients were not being treated with antibiotics when this coagulopathy developed, and we do not understand interferon's apparent effect on vitamin K. The abnormalities of factor XII cannot be explained by an effect on vitamin K, and the mechanism of interferon's effect on this factor also remains unknown. Disseminated intravascular coagulation apparently did not occur since factors V and VIII were normal when measured. Although the liver-synthesized factors (II, VII, IX, and X) were low, decreased hepatic synthesis did not appear responsible for this coagulopathy, since the fibrinogen remained normal in six of the seven patients. Additionally, factor V (liver-synthesized) was normal in Patient 4 during the interferon infusion (109%, table 2), while the APTT was abnormal (60.2 seconds, table 1).

Unfortunately, coagulation factors were retrospectively analyzed and a prospective study will be required to elucidate the biochemical mechanisms responsible for this coagulopathy. Investigators using high-dose interferon should be aware of this coagulopathy and monitor patients carefully.

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Supporting Evidence for an Active Treatment Program for Advanced Salivary Gland Carcinomas[1]

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The role of systemic chemotherapy in the treatment of salivary gland tumors remains unknown. Because of the infrequent occurrence of these tumors, only a few clinical trials with chemotherapy have been reported in the literature. The current trial presents preliminary results with the combination of cyclophosphamide, doxorubicin, and cisplatin. These results are viewed in terms of other recently reported data obtained with the same combination. The collected data strongly suggest that salivary gland tumors may be very responsive to chemotherapy. [Cancer Treat Rep 69:319–321, 1985]

Salivary gland tumors are rare neoplasms, representing < 5% of all head and neck tumors (1). The primary mode of therapy is surgery, but frequent locoregional recurrences often necessitate additional operations. Postoperative radiotherapy is frequently applied in an attempt to decrease the incidence of recurrence in patients at high risk (1). Because of the uncommon occurrence of these tumors, experience with chemotherapy is limited, and reports in the literature are confined to small series. However, evidence is accumulating that salivary gland tumors may, in fact, be very responsive to systemic chemotherapy (2–5).

This is a preliminary report of a pilot experience with cyclophosphamide, doxorubicin, and cisplatin (CAP) chemotherapy in unselected patients with advanced incurable salivary gland carcinomas. Eligibility criteria included the presence of measurable disease, Eastern Cooperative Oncology Group performance status of 0–3, and appropriate renal and hematological parameters; the response criteria were those of Creagan et al (2). Cisplatin was given at doses of 60 mg/m^2 in 100 ml of 5% dextrose in one-half normal saline over 1 hour every 3 weeks, with saline hydration and mannitol-induced diuresis (two patients) or at weekly outpatient doses of 20 mg/m^2 in 150 ml of one-half normal saline without diuresis (two patients). Cyclophosphamide (400 mg/m^2) and doxorubicin (40 mg/m^2) were given iv every 3 weeks.

RESULTS AND DISCUSSION

The results for the initial four patients entered thus far in this trial are shown in table 1. Although the significance of the preliminary data is obviously limited, a brief review of recently published studies with CAP in salivary gland tumors reveals interesting results. Creagan et al (2) reported two complete remissions and six partial remissions among 16 patients with non-squamous cell carcinomas of the head and neck, including five primaries from the parotid gland (three adenocarcinomas and two acinic cell carcinomas), one adenoid cystic carcinoma of the submaxillary gland, one adenocarcinoma in the nasopharynx, and one apocrine sweat gland carcinoma of the scalp. Most patients had failed prior radiotherapy and/or presented with locoregional or distant metastatic disease (pulmonary metastasis). Alberts et al (3) observed responses in all five of their patients with parotid gland cancer, including two complete remissions. Histologic types included three adenocarcinomas and two tumors of mixed histology. Responses in all three studies were observed, both locoregionally and in distant metastatic sites.

Thus, when our results are combined with the existing data in the literature, 17 of 25 patients with advanced disease have responded to chemotherapy with CAP. Furthermore, seven of these responders achieved complete remissions persisting for 1, 3, 4.6, 7, 7, 11+, and 14.6 months. These results indicate that this is a promising treatment program for this disease. However, since this conclusion is based on a collection of patients from various series using different dose schedules, it should be cautiously interpreted, and the findings should be reproduced by larger multi-institutional cooperative groups

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TABLE 1.—Preliminary results with CAP chemotherapy in salivary gland tumors

Patient age and sex	Stage*	Disease status and histology	Prior therapy	Response to chemotherapy†	Duration of Response (mos)	Survival (mos)	Comments
57-yr-old female	$T_4N_{3B}M_0$	"De novo" presentation. Adenocarcinoma of the parotid gland.	None (unresectable lesion)	Clinical CR (*see* comments)	3	5	Primary lesion (12 × 7 cm) completely regressed following 3 courses of CAP, leaving large necrotic fistula subsequently closed surgically with cutaneous flap. Pathologically, the margins showed persistent tumor. Received 3 more courses of CAP, resulting in no palpable evidence of disease. Died with neutropenic sepsis. At autopsy no evidence of tumor was found.
51-yr-old female	$T_4N_{3A}M_0$	Regional (lymph nodes) recurrence. Mucoepidermoid carcinoma of the parotid gland.	Surgery and radiotherapy (7000 rad)	Clinical CR	11+ (6+ of unmaintained CR)	12+	Recurrent neck node was unresectable due to right carotid encasement. Carotid pulse became normal following clinical CR. Only moderate hematological toxicity and nausea and vomiting.
73-yr-old male	$T_4N_1M_0$	Locoregional recurrence. Mucoepidermoid carcinoma of the soft palate with extension to the right maxillary sinus.	Radiotherapy (7000 rad), methotrexate, bleomycin	Excellent PR	9	11+	Marked symptomatic improvement. Moderate hematological toxicity and nausea and vomiting.
57-yr-old male	$T_xN_0M_1$	Distant metastasis (lung) only. Mucoepidermoid carcinoma of the floor of the mouth.	Surgery and radiotherapy (5400 rad)	Clinical CR on chest X-ray and lung tomograms	7 (had local recurrence in primary site) (*see* comments)	10+	Only clinical evidence of disease at the time of study entry was on chest x-ray. Following local recurrence, chest x-ray remained negative for total of 10 mos.

* Stage at time of entry in this study.
†CR = complete remission; PR = partial remission.

with a larger number of patients.

The results with single agents are less defined, and this issue needs to be addressed, since it is possible that the activity of the CAP combination may be due to only one of its components. The results of Schramm et al (4), who obtained seven responses among 10 patients treated with cisplatin, as well as preliminary data (5,6) showing that doxorubicin also may have some activity in this disease, further suggest the need to clearly define the role of each single agent in the CAP regimen.

The preliminary data indicate important leads that warrant further systematic development of this chemotherapeutic program in a disease where little is known about the efficacy of this treatment modality.

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Phase I Clinical Evaluation of Diaziquone in Childhood Cancer[1,2]

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Diaziquone (AZQ), a new lipid-soluble antitumor agent, was given by 15–30-minute infusion on a daily × 5 schedule to 47 children with refractory solid tumors and leukemia. The starting daily dose of 6 mg/m² was escalated to 10 and 35 mg/m² in patients with solid tumors and leukemia, respectively. In patients with solid tumors, myelosuppression was dose-limiting at a daily dose of 10 mg/m². In patients with leukemia, prolonged pancytopenia and bone marrow hypoplasia were observed at daily doses ≥ 25 mg/m². At these higher doses, significant hyperbilirubinemia associated with sepsis was also seen. Corresponding increases of transaminases or alkaline phosphatase and significant hemolysis were not noted. The maximum tolerated dose for this daily dose schedule was 9 mg/m² in children with solid tumors and 25 mg/m² in children with relapsed leukemia. Responses to AZQ included stabilization of disease in osteosarcoma, neurofibrosarcoma, pinealoma, and ependymoma. A patient with juvenile chronic myelocytic leukemia in blast crisis converted back to the chronic phase. A patient with acute lymphoblastic leukemia had a substantial decrease in cerebrospinal fluid blast count. Bone marrow aplasia was achieved in children with acute lymphoblastic leukemia and acute nonlymphoblastic leukemia; however, remissions were not achieved. A phase II study of AZQ in children with refractory malignancies is now being performed by the Childrens Cancer Study Group. [Cancer Treat Rep 69:323–327, 1985]

Diaziquone (AZQ) is an antitumor agent in which the molecular characteristics of lipid solubility, low ionization, and water solubility are optimized for entry in the central nervous system (1). It has a broad spectrum of antitumor activity in experimental tumor systems (1,2). In adult and pediatric phase I trials and preliminary reports of adult phase II trials, AZQ has demonstrated activity against primary brain and spinal cord tumors (1,3–9), non-Hodgkin's lymphoma (10,11), Hodgkin's disease (9,10), and acute and chronic leukemia (9,12,13). Myelosuppression, in particular thrombocytopenia, was the dose-limiting toxic effect.

Based on the activity of AZQ in preclinical and adult phase I studies, we undertook a phase I study of AZQ in children. Details of the clinical study are reported here. The pharmacologic disposition study is the subject of a separate paper.

MATERIALS AND METHODS

Eligibility Criteria

Patients < 18 years of age with pathologic confirmation of malignancy that was resistant to conventional therapy were eligible for study. In the judgment of the attending physician, an estimated life expectancy of at least 12 weeks was required. All patients had measurable disease, although this was not a prerequisite for entry in the study. All patients were required to have adequate bone marrow function (peripheral absolute neutrophil

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†Deceased. This study is dedicated to the memory of Gussie R. Higgins, MD.

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count > 1000/mm^3 and platelet count > 100,000/mm^3), with the exception of patients with leukemia or with solid tumors accompanied by bone marrow involvement. Adequate liver (bilirubin < 2.0 mg/dl) and renal (creatinine < 1.5 mg/dl) functions were required. In addition, patients must not have received any previous chemotherapy and/or radiotherapy for at least 4 weeks, unless there was obvious tumor progression, and the patient must have recovered from any toxic effects of prior therapy. Signed informed consent indicating the investigational nature of this study was obtained in accordance with federal and institutional guidelines.

Drug Formulation and Administration

AZQ was supplied by the National Cancer Institute (Bethesda, MD) as a sterile powder in amber vials containing 10 mg of the compound. For clinical use, it was reconstituted with 0.5 ml of sterile *N,N*-dimethylacetamide. This solution was further diluted with 9.5 ml of sterile 0.01 M, pH 6.5, phosphate buffer and then with lactated Ringer's solution or sodium chloride injection, USP, to a concentration of 0.5 mg/ml. This solution was administered iv over 15–30 minutes.

The initial dose of AZQ was 6 mg/m^2/day for five daily doses to be repeated every 3 weeks. This dose was approximately 80% of the maximum tolerated dose as determined for adult patients. Dosage escalation was by 1-mg/m^2 increments in patients with solid tumors to a maximum of 10 mg/m^2 daily for 5 days. In patients with leukemia, following the 10-mg/m^2 dose level escalations were to 20, 25, 27.5, 30, and 35 mg/m^2/dose. There was no dose escalation in individual patients. At least three patients were treated at each dose level before dose escalation was carried out. Patients were treated for at least two cycles of therapy, unless tumor progression or unacceptable toxicity occurred.

Patient Monitoring During Study

An interval history and physical examination were required prior to each course of therapy. Cbc with platelet count and differential, urinalysis, and biochemical profile were performed weekly. Studies to determine tumor response, such as bone marrow examinations or radiologic studies, were performed every 3–8 weeks. Patients were considered evaluable for toxicity if they received at least one course of therapy with 2 weeks of subsequent observation. The toxicity rating scale of the Childrens Cancer Study Group was utilized in this study; grade 3 and 4 toxicity is considered to be significant. Of relevance to the nonhematologic toxicity reported herein, significant hepatotoxicity was defined as transaminase, alkaline phosphatase, and total bilirubin greater than or equal to five, two, and two times normal, respectively. Standard criteria of response were used to evaluate the antitumor effect of the drug.

RESULTS

Patient Characteristics

Forty-seven patients (19 female and 28 male, ranging in age from 1 year and 5 months to 16 years and 10 months [median, 8 years and 4 months]) were entered and eligible for this study. Three additional patients were ineligible and are therefore excluded from further analysis. All eligible patients had pathologic confirmation of malignancy that was resistant to conventional therapy. One patient had previously been treated with radiotherapy alone, three had received chemotherapy alone, and 43 had received combined modality therapy. A median of three prior chemotherapeutic regimens (range, none to seven) had been administered. The spectrum of diagnoses is listed in table 1.

Nonhematologic Toxicity

Sixty-six courses of AZQ administered to 47 patients were evaluable for nonhematologic toxicity. Mild, non-dose-related nausea, vomiting, and stomatitis were seen. A single patient developed diarrhea at the 10-mg/m^2 dose. Lethargy was reported during three of ten evaluable courses of AZQ at the 30- and 35-mg/m^2 dose levels. Other causes of lethargy in these patients could not be definitely excluded. Most patients had alopecia at study entry; however, only at doses ≥ 25 mg/m^2 could AZQ-induced alopecia be documented.

The major nonhematologic toxic effect of AZQ was hepatic (table 2). There was mild to moderate transaminase elevation at all doses. In one patient each at the 10-, 25-, 30-, and 35-mg/m^2 dose levels, a direct bilirubin ≥ 5.0 mg/dl was documented. In these patients, the transaminases and serum alkaline phosphatase were not concomitantly increased and increased hemolysis could not be documented. Each of these episodes was associated with either proven or suspected bacterial or fungal sepsis. Two patients developed hyperbilirubinemia shortly prior to their death. At postmortem examination, hepatic congestion and bile stasis were seen in one patient with a total bilirubin of 13.9 mg/dl and mild central por-

TABLE 1.—Diagnoses of study patients

Diagnosis	No. of patients
Acute lymphoblastic leukemia (ALL)	11
Non-Hodgkin's lymphoma with leukemic transformation	1
Acute nonlymphoblastic leukemia (ANLL)	9
Chronic myelocytic leukemia (CML) in blast crisis	2
Juvenile CML in blast crisis	1
Neuroblastoma	5
Wilms' tumor	3
Hepatoblastoma	2
Miscellaneous sarcomas	6
Brain tumors	7

TABLE 2.—Hepatic toxicity

Dose (mg/m^2)	No. of courses: Evaluable	No. of courses: Without toxicity	No. of courses: With toxicity	Hepatic toxicity by individual course of treatment*: SGOT (units/L)	SGPT (units/L)	Alkaline phosphatase (units/L)	Total/direct bilirubin (mg/dl)	Comments
6–10	27	17	10	803	250	72	0.5/—	Normalized within 1 wk
				24	15	140	4.4/—	Normalized within 1 wk
				34	126	60	0.5/—	
				53	202	52	0.5/—	
				56	213	54	0.6/—	
				14	14	79	1.4/—	
				138	24	109	1.7/—	Bilirubin normalized within 1 wk
				32	53	210	0.1/—	
				43	163	55	0.7/—	
				164	160	52	5.0/—	Associated with *Enterobacter cloacae* sepsis
20–25	12	7	5	62	98	85	0.6/—	
				88	123	136	0.5/—	
				137	143	145	0.7/—	
				90	45	177	2.7/—	
				32	28	38	5.1/4.2	Associated with *Escherichia coli* sepsis
27.5–35	12	5	7	25	13	48	1.5/—	Associated with *Enterobacter cloacae* sepsis and *Candida* liver abscesses
				63	106	112	0.4/—	
				18	31	334	1.0/—	
				83	82	128	0.8/—	
				4	29	91	13.9/10.8	Associated with *Candida albicans* sepsis during 2nd course of AZQ; congestion and bile stasis at autopsy
				98	170	190	12.0/8.5	Associated with sepsis
				7	12	161	3.0/2.2	Associated with *Escherichia coli* sepsis during 2nd course of AZQ; mild central portal fibrosis and marked central vein congestion at autopsy

* Normal values: SGOT (15–55 units/L); SGPT (3–37 units/L); alkaline phosphatase (30–280 units/L); and total/direct bilirubin (< 1.2/< 0.5 mg/dl).

tal fibrosis and marked central vein congestion were seen in the other patient with a total bilirubin of 2.7 mg/dl.

Hematologic Toxicity

Myelosuppression was the dose-limiting toxic effect of AZQ. Dose escalation and occurrence of hematologic toxicity for the 17 patients without documented bone marrow involvement are summarized in table 3. While the degree of myelosuppression was progressively more pronounced at the higher dose levels, severe leukopenia, neutropenia, thrombocytopenia, and anemia were noted at all dose levels. The time to leukopenia (wbc count $< 2500/mm^3$), neutropenia (absolute neutrophil count $< 1000/mm^3$), and thrombocytopenia (platelet count $< 100{,}000/mm^3$) and the time to the nadir of these blood cell counts was generally between 2 and 3 weeks from the start of a course of therapy and was not dose-dependent. Recovery usually occurred within 1–2 weeks, although several patients had extremely prolonged periods of cytopenias. No deaths could be attibuted to myelosuppression in patients with solid tumors.

Except for the period of bone marrow aplasia, myelosuppression could not be evaluated in patients with leukemia. Twenty-seven courses of AZQ were administered to 19 patients at daily doses $\geq 20\ mg/m^2$. Seven patients who developed severe pancytopenia had bone marrow examinations performed between Days 17 and 25 of study. All of these bone marrow specimens were aplastic, although three still had identifiable leukemic cells. Six patients were severely pancytopenic without peripheral blasts between Days 18 and 34 of therapy; however, bone marrow examinations were not performed in these patients. A single patient with juvenile CML had a normocellular remission bone marrow on Day 27; recovery of the neutrophil count to $> 1000/mm^3$ and of the platelet count to $> 100{,}000/mm^3$ did not occur until Day 34.

Nine deaths occurred in the 19 patients treated at daily doses of $\geq 20\ mg/m^2$ following the first course of AZQ. In the patient who died on Day 8, a postmortem bone marrow biopsy was severely aplastic. A second patient died on Day 34 while still severely pancytopenic without circulating blasts; a bone marrow examination was not performed. The remaining seven patients died between Days 17 and 36 with either an M3 bone marrow or circulating blasts. Two of eight patients who received two courses of

TABLE 3.—Hematologic toxicity in patients without documented bone marrow involvement

Dose (mg/m^2)	No. of patients entered	No. of evaluable courses	Median wbc count nadir, $\times 10^3$ cells/μl (range)	Median absolute neutrophil count nadir, $\times 10^3$ cells/μl (range)	Median platelet count nadir, $\times 10^3$ cells/μl (range)	Median fall in hemoglobin, g/dl (range)
6	2	4	5.3 (0.9-5.7)	4.2 (0.4-4.8)	155 (< 5-214)	2 (0-4)
7	2	2	2.7 (1.4-3.9)	1.4 (1.1-1.7)	69 (51-87)	1.5 (0-3)
8	5	9	4.4 (0.6-6.1)	3.0 (0.3-5.1)	176 (< 5-287)	0 (0-5.7)
9	5	11	2.0 (1.3-6.1)	1.3 (0.3-5.2)	77 (6-192)	1.7 (0-3.3)
10	3	2	1.0 (0.7-1.3)	0.4 (0-0.8)	46 (14-77)	3.2 (2.6-3.8)

AZQ died with aplastic bone marrow specimens on Days 17 and 21 of the second course of therapy. Three additional patients died between Days 13 and 41 of the second course of therapy with either an M3 bone marrow or circulating blasts.

Therapeutic Activity

No patient with a solid tumor had a complete or partial response. Stable disease was seen in one patient each with osteosarcoma (100 days), pinealoma (100 days), and neurofibrosarcoma (61 days) and in two patients with ependymoma (113 and 132 days).

Oncolytic responses or bone marrow aplasia were achieved in 11 of 19 evaluable patients with ALL and ANLL; however, all patients either had return of blasts or died of sepsis prior to bone marrow recovery. One patient with ALL treated at the 6-mg/m^2 dose level had a decrease in cerebrospinal fluid blast count from 744 to 27/mm^3 by Day 5 of AZQ (14). One of two patients with CML in blast crisis had a three-log reduction in peripheral wbc count. One patient with juvenile CML in blast crisis converted back to the chronic phase following one course of AZQ at the 35-mg/m^2 dose level. Following a second course of therapy of 50% of the induction dosage, the patient died from multifocal hemorrhages and candidiasis while severely pancytopenic.

DISCUSSION

This study demonstrates that the dose-limiting toxic effect is myelosuppression for pediatric patients with solid tumors receiving AZQ and that the maximum tolerated dose is 9 mg/m^2/day when given by rapid infusion on a 5-day schedule.

In patients with leukemia, the dose-limiting toxic effect was severe prolonged bone marrow suppression. The maximum tolerated dose in patients with leukemia in relapse was 25 mg/m^2/day for 5 consecutive days. Marked hyperbilirubinemia was seen in association with sepsis at these higher doses. The clinical and pathologic findings suggested that this latter finding was related to sepsis that occurred in association with profound neutropenia.

The time to onset and nadir of bone marrow suppression, as documented by peripheral blood cell counts, was 2-3 weeks after initiation of therapy. This nadir occurred somewhat later than with other chemotherapeutic agents, with the exception of the nitrosoureas. Bone marrow recovery also was quite prolonged in several patients. In general, thrombocytopenia appeared to be more prolonged than neutropenia.

AZQ was otherwise well-tolerated by all patients. Transient transaminase elevation occurred in almost one-half of the patients. Nausea, vomiting, diarrhea, and mucositis occurred infrequently and were generally of a minor degree.

The toxic effects seen in this pediatric study are quite similar to those reported from studies in adults (1,15,16). Several different dosage regimens have been studied in adults, but when given on a 5-day schedule as administered in this study, the maximum tolerated doses for patients with solid tumors and leukemias were 4-8 and 20-25 mg/m^2/day, respectively (1,3,4,12,15).

Although no complete or partial responses were seen in our study, stable disease occurred in three patients with brain tumors and in one each with osteosarcoma and neurofibrosarcoma. This confirms more extensive data in the adult population with brain tumors in which objective responses have been reported (1,3-9). Therapeutic activity has also been documented in non-Hodgkin's lymphoma in adults (10,11) and Hodgkin's disease in children and adults (9,10).

Remissions were not achieved in patients with acute leukemia; however, therapeutic activity of AZQ was demonstrated by the attainment of bone marrow aplasia in ALL and ANLL. Tan et al (9), using an identical dosage schedule, described two complete and three partial responses in 15 evaluable children with ANLL treated at a daily dose of either 25 or 30 mg/m^2. One patient in our study with ALL had a significant decline in the cerebrospinal fluid blast count on Day 5 of AZQ (14), confirming a complete response of meningeal leukemia to iv AZQ described by Doering (13). A single patient in our study with juvenile CML in blast crisis converted back to the chronic phase following one course of AZQ.

On the basis of these toxicity data and on the evidence of antitumor activity in this and other studies, we recommend that phase II trials be initiated in children with refractory malignancies. The recommended dose is 9 mg/m^2 daily for 5 days in children with solid tumors, repeated

every 3–4 weeks. In children with leukemia, the recommended induction dose is 25 mg/m^2 daily for 5 days. The maintenance dose recommended is 9 mg/m^2 daily for 5 days repeated every 3–4 weeks and is reduced to avoid the severe and prolonged myelosuppression that would be anticipated from the studies of patients with solid tumors without bone marrow involvement.

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Phase II Trial of Mitogauzone in Malignant Primary Brain Tumors[1,2]

Lynn G. Feun,[3] Wai-Kwan A. Yung,[3] David J. Stewart,[4] Niramol Savaraj,[3] and Gerald P. Bodey[3]

Sixteen patients with primary malignant brain tumors, recurrent or progressive after surgery and radiation therapy, were treated with mitoguazone. Starting dose was 500 mg/m² iv on a weekly schedule. In 15 evaluable patients no tumor regressions were observed. Four patients had stabilization of disease. [Cancer Treat Rep 69:329–330, 1985]

Pharmacological studies have demonstrated that when mitoguazone (MGBG) is administered iv, potentially cytotoxic drug levels can be achieved in brain tumors (1). MGBG appears to inhibit *s*-adenosyl methionine decarboxylase, an important enzyme in polyamine synthesis (2), although other mechanisms of action may be involved. Polyamines may be important modulators of cell growth. Inhibition of DNA synthesis following MGBG is associated with depletion of spermidine and spermine and increases in putrescine. In a recent report, the polyamine levels in a rat brain tumor in vitro appeared to correlate with the specific growth rate (3). Elevated cerebrospinal fluid levels of polyamines have been reported in patients with primary brain tumors (4).

For these reasons, a clinical trial of MGBG was undertaken in patients with primary malignant brain tumors progressing after surgery and cranial radiation therapy.

MATERIAL AND METHODS

Sixteen patients were entered into the study; 15 had histologically confirmed primary malignant brain tumors (table 1). One patient had a presumed brain stem glioma. Twelve patients had received prior chemotherapy: nitrosoureas (11 patients), cisplatin (six), diaziquone (two), etoposide (two), and vincristine (one). At the time of therapy with MGBG, all patients had evidence of tumor recurrence and/or progression documented by computed tomographic (CT) brain scan and clinical neurological examination. The eligibility requirements for entry into this study were unequivocal deterioration attributable to tumor regrowth, no surgery for at least 1 month, no radiotherapy or chemotherapy for at least 3 weeks (6 weeks for nitrosoureas), and normal cbc, platelet count, SMA 12, and urinalysis. Informed consent was obtained from each patient and/or responsible next of kin before drug therapy was started.

MGBG was started at an initial dose of 500 mg/m² for all of the patients. The drug was given as a single injection in 100 ml of 5% dextrose in water or normal saline and infused iv over 30–60 minutes. Courses were given at weekly intervals as tolerated. MGBG was decreased in patients who developed severe marrow toxic reactions (lowest granulocyte count $< 1000/mm^3$ and/or lowest platelet count $< 60{,}000/mm^3$) or unacceptable myalgia or mucositis. An attempt to increase the dosage by 20% was made if the patient had received four courses with no significant morbidity.

Patients were evaluated by a neurological examination and CT scan repeated monthly. The results were compared with the preceding tests. Response was defined as definite improvement in both the neurological status and CT scan while receiving a stable or decreasing dose of steroids. Progression was defined as deterioration in both tests while receiving a stable dose of steroids. Stable disease was defined as no change in the tests while receiving a stable or decreasing dose of steroids.

RESULTS

Of the 16 patients who received MGBG chemotherapy, one patient was considered inevaluable. This patient had an early death due to progressive pulmonary fibrosis from previous treatment with carmustine. The remaining 15 patients received a median of four courses of MGBG (range, one to 11). There were no complete or partial re-

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TABLE 1.—Patient characteristics

Characteristics	No. of patients
Entered	16
Inevaluable	1
Median age in years (range): 46 (23-64)	
Sex	
Male	8
Female	7
Diagnoses	
Glioblastoma	10
Recurrent low-grade astrocytoma	1
Medulloblastoma	1
Pineoblastoma	3
Prior therapy	
Surgery	15/15
Radiotherapy	15/15
Chemotherapy	12/15
Median performance status (range): 2 (1-3)	

sponses. Four patients had stabilization of disease; the diagnoses were glioblastoma (two patients), brain stem glioma (one), and pineoblastoma (one). For these patients the median time to tumor progression was 6 months (range, 3-10). Nine patients had progressive disease.

Hematological toxicity was mild. Two patients (13%) each developed significant thrombocytopenia (platelet count $< 70{,}000/mm^3$) and leukopenia (wbc count $< 1000/mm^3$). However, all four patients were heavily pretreated with chemotherapy. Other toxic effects included mild hyperglycemia in one patient, moderate stomatitis in one, and pulmonary embolism in two. Moderate myalgia and dermatitis in two patients necessitated a reduction in the dosage of MGBG.

DISCUSSION

It is disappointing that in this study no definite tumor regressions were demonstrated after treatment with MGBG. In addition, only four of 15 evaluable patients with recurrent cerebral neoplasms exhibited any stability of disease. Therefore, it appears that MGBG by itself, in this schedule, may be ineffective in the treatment of recurrent malignant primary brain tumors.

It may be possible to achieve better results using a combination of MGBG with α-difluoromethylornithine (DFMO). DFMO is a new agent which irreversibly inhibits the rate-limiting reaction in polyamine synthesis (5). Experiments have shown that the combination of MGBG with DFMO produces greater cytotoxicity than either drug used alone (6,7). Also, the addition of MGBG or DFMO to carmustine potentiated the antitumor therapeutic effects of carmustine in intracerebral tumors of experimental animals (8,9). The actual role of polyamine inhibitors in the treatment of malignant brain tumors remains to be elucidated.

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Diaziquone for Resistant Multiple Myeloma[1,2]

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For the Cancer and Leukemia Group B

Current recommended treatment for symptomatic multiple myeloma consists of combination chemotherapy with melphalan and prednisone (1) or with multiple alkylating agents (2). Once patients fail standard therapy, remissions with salvage treatment occur infrequently. Diaziquone (AZQ) is a lipophilic aziridinylbenzoquinone that has completed phase I and entered phase II trials (3). AZQ is believed to possess alkylating activity which makes it an ideal drug to test in multiple myeloma. The Cancer and Leukemia Group B carried out a pilot study to test the effectiveness of AZQ in the treatment of refractory multiple myeloma.

Criteria for the diagnosis of multiple myeloma were previously reported (4). Patients who had received prior therapy and had not achieved response or had relapsed were eligible for this study. Patients were required to have anticipated survival > 2 months, normal SGOT or SGPT, and no evidence of bone marrow depression from prior chemotherapy or radiotherapy.

AZQ was dissolved in 100 ml of normal saline and infused over a period of 30 minutes every 3 weeks. The dose for the first cycle was 30 mg/m^2 and was increased to 35 mg/m^2 for the second cycle if the wbc count was > 3500/μl and platelet count was > 100,000/μl. Patients were monitored with weekly cbc and platelet count and with serum liver function tests, creatinine, BUN, and glucose every 21 days.

Thirty-six patients were entered on study. The pretreatment patient entry characteristics and toxic effects are listed in table 1. Nine patients remain alive at last report. Of the 36 patients on study, 12 (33%) received the full dose of AZQ, 22 (61%) received between 50% and 100% of the full dose, and two (6%) received < 50% of the prescribed dose. The reason that only one-third of the patients received the full prescribed dose of AZQ was due to dose modifications for excessive hematologic toxicity after the first course of treatment. Approximately one patient in five had life-threatening platelet toxicity.

Patient response to therapy was judged by Chronic Leukemia-Myeloma Task Force criteria (4). None of the patients achieved response or improved status. Of the 36 patients, ten were stable, 23 had progressive disease, and three died while on study without showing improvement. The median survival for all patients was 3.5 months.

In this study of heavily pretreated patients with multiple myeloma, AZQ did not demonstrate any therapeutic activity. Toxicity was primarily hematologic, with life-

TABLE 1.—Patient entry characteristics and toxic effects

Characteristics	No. of patients (%)	
Sex		
Female	14 (39)	
Male	22 (61)	
Age		
40–49	1 (3)	
50–59	12 (33)	
60–69	13 (36)	
70+	10 (28)	
Initial performance status		
1	5 (14)	
2	9 (25)	
3	11 (31)	
4	11 (31)	
Prior single-agent chemotherapy	17 (47)	
Prior combination chemotherapy	34 (94)	
Prior radiotherapy	18 (50)	
Mean No. of cycles of therapy: 2.1		
Toxic effects	*Severe*	*Life-threatening*
Hemoglobin	1 (3)	1 (3)
Wbc count	9 (25)	2 (6)
Platelet count	8 (22)	7 (19)
Infection	5 (14)	0 (0)
Hemorrhage	2 (6)	0 (0)
Nausea, vomiting	0 (0)	1 (3)
Renal	2 (6)	0 (0)

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threatening thrombocytopenia seen in about 20% of patients. Other new agents should be tested for clinical efficacy.

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Phase II Evaluation of Amsacrine in Small Cell Lung Cancer: A Southeastern Cancer Study Group Trial[1,2]

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Amsacrine (*m*-AMSA) is one of a series of acridine derivatives which inhibits DNA replication by binding to DNA (1). Phase I investigations suggested activity in lung adenocarcinoma (2).

We therefore performed a phase II study in previously treated patients with extensive small cell lung cancer who fulfilled the following criteria: (*a*) diagnosis confirmed by histology; (*b*) at least one evaluable lesion; (*c*) Karnofsky performance status ≥ 40%; (*d*) recovery from previous myelosuppressive therapy; and (*e*) signed informed consent. Amsacrine was given at a dose of 40 mg/m^2 iv in 250 ml of 5% dextrose in water by 1-hour infusion on each of 3 successive days every 3 weeks. The dose was reduced to 20 mg/m^2 in the four patients with initial abnormal liver function tests.

Of 21 patients (median Karnofsky performance status of 70%; range, 60%-90%) entered in the study, 20 were evaluable for response. One patient was inevaluable because of failure to meet the eligibility criteria for study entry. There were no responses in the remaining 20 patients, 14 of whom had progressive disease and two no change. In addition, there were three early deaths, and one patient refused treatment after one course. Median survival in evaluable patients was 12.4 weeks. The 95% confidence interval for zero of 20 responses is 0%-14%. The median number of courses was two (range, one to four).

Drug-related toxicity was primarily hematologic (table 1). Six patients developed granulocyte count nadirs < 1000/mm^3; three of them < 500/mm^3. Four patients had platelet count nadirs between 20,000 and 75,000/mm^3. One patient had central nervous system toxicity of disorientation. There were no other significant toxic effects and no drug-related deaths.

There was no activity in this study against small cell lung cancer using a daily × 3 dose every 3 weeks. Our findings agree with those of other phase II studies (3-5) which used a different dose schedule (a single dose every 3 weeks). Amsacrine should no longer be investigated in previously treated small cell lung carcinoma.

TABLE 1.—Hematologic toxic effects[*]

	Granulocyte count		Platelet count	
Toxicity grade	No. of patients	Nadir range (cells/mm^3)	No. of patients	Nadir range (cells/mm^3)
0	9	2100-7200	11	185,000-427,000
1	3	1600-1890	2	100,000
2	1	1300	2	78,000-87,000
3	0	—	4	20,000-67,000
4	6	64-900	0	—

* Nadir counts not recorded in one case.

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[2]Supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: The University of Florida, Gainesville (*B. S. Kramer*, CA-28143); Southeastern Cancer Study Group Statistical Center, University of Alabama in Birmingham (*R. Birch*, CA-24456); Indiana University School of Medicine, Indianapolis (*L. Einhorn*, CA-28171); and Vanderbilt University School of Medicine, Nashville, TN (*A. Greco*, CA-23909). Other participating institutions include: Brooklyn Veterans Administration Hospital, NY; City of Hope Medical Center, Duarte, CA; University of Cincinnati, OH; Duke University, Durham, NC; University of Kentucky, Lexington; University of Puerto Rico, San Juan; Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL; and University of Tennessee, Memphis.

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Pediatric Oncology Group Phase II Trial of Amsacrine in Children With Solid Tumors[1,2]

Curt I. Civin,[3] Jeffrey P. Krischer,[4] Vita J. Land,[5] Ruprecht Nitschke,[6] Barton Kamen,[7] and Tribhawan Vats[8,*]

Amsacrine (AMSA) is an acridine derivative active in adult (1) and childhood (2) leukemia. Despite broad antitumor activity in preclinical studies (3), AMSA has no major activity against adult solid tumors. AMSA was tested against pediatric solid tumors, using a dose with antileukemic efficacy.

PATIENTS AND METHODS

Children were eligible if their tumors (measurable, relapsed) were resistant to standard therapy; their renal, hepatic, and cardiac functions were normal; they had received no prior AMSA and no concurrent antineoplastic therapy; and they (or their parents) consented to study participation.

Patients received AMSA at 60–75 mg/m^2 daily for 5 days at 21-day intervals (or at full hematopoietic recovery), with a 20% decrease if the previous course had resulted in granulocytopenia (< 500 cells/μl).

Complete response (CR) was defined as disappearance of all clinical tumor. Partial response (PR) was defined as > 50% reduction in the largest diameter of each tumor. Response was determined 3 weeks after one (in cases of progressive disease in 15 patients) or two AMSA courses.

RESULTS AND DISCUSSION

Three of 54 accrued patients were ineligible and three were inevaluable (two early deaths and one insufficient data). Of 48 evaluable patients, none achieved CR. There were three PRs, all in patients who had neuroblastoma (table 1). PRs were brief (7, 22, 30, and 35 days).

AMSA toxic effects (table 2) were as described previously (2). Severe neutropenia (< 500 neutrophils/μl) and thrombocytopenia (< 50,000 platelets/μl) of 1–3 weeks' duration were observed in most cases (with three deaths due to infections and one to pulmonary hemorrhage); five cases of cardiac arrhythmia were observed.

TABLE 1.—Response to AMSA in children with solid tumors in relapse*

	Histiocytosis X	Wilms' tumor	Neuroblastoma	Osteosarcoma	Other†	Total
PR‡	0	0	3	0	0	3
Mixed response‡	1	0	2	0	0	3
No response	0	0	2	0	3	5
Progressive disease	0	3	10	7	17	37
Total	1	3	17	7	20	48

* At first, patients received AMSA at 75 mg/m^2 daily for 5 days. When this dose was found to result in unacceptable myelotoxicity, it was lowered to 60 mg/m^2 daily (for 14 patients). Values in table = No. of patients.

†Other tumors included Ewing's sarcoma (8 patients), rhabdomyosarcoma (5), soft tissue sarcoma (2), fibrosarcoma (2), retinoblastoma (1), astrocytoma (1), and hepatocellular carcinoma (1). No responses were observed.

‡3 of 3 mixed responses and 2 of 3 PRs occurred at the 75-mg/m^2/day dosage. *See* the Results and Discussion section for response durations.

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TABLE 2.—Toxic effects of AMSA

Toxic effect	% of patients
Granulocytopenia (< 500 cells/μl)	81
Thrombocytopenia (< 50,000 cells/μl)	73
Anemia (Hgb < 7 g/dl)	35
Cardiac arrhythmia	10
Nausea and vomiting	12
Rash	4
Venous sclerosis (phlebitis)	4
Mucositis	2

Thus, AMSA demonstrated minimal clinical activity, even in neuroblastoma, with no CRs and only brief PRs or mixed responses. No remarkable activity was noted in other pediatric solid tumors, although the study was closed (due to toxicity) before enough cases were accrued to reach this conclusion for *each* type of pediatric solid tumor.

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Phase II Evaluation of Bisantrene in Metastatic Breast Cancer[1]

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Bisantrene is a new anthracene derivative which is undergoing extensive phase II evaluation. In the human tumor cloning assay, the drug exhibited promising antitumor activity, especially against breast cancer (1,2). This report describes the final results of a phase II trial conducted between April 1982 and February 1983 by the Early Clinical Trials Group of the European Organization for Research on Treatment of Cancer.

PATIENTS AND METHODS

Fifty-five patients with measurable metastatic breast cancer were entered in the study. Two patients were ineligible and four were not evaluable (lost to follow-up, one; early death at ⩽ 4 weeks, three). Pretreatment characteristics of the 49 evaluable patients are summarized in table 1.

Bisantrene was administered as a single dose repeated every 3 weeks. Initially, patients who were considered poor-risk (extensive prior radiotherapy and/or four or more prior cytotoxic drugs) were given 240 mg/m^2; all other patients received 280 mg/m^2. Doses were escalated by a single increment of 20 mg/m^2 when the wbc count nadir in the previous course was ⩾ 4000/mm^3 (15 patients). Among the last 16 entries, poor-risk patients were started at 260 mg/m^2 and the others at 300 mg/m^2. Bisantrene was infused over 1 hour via a central catheter (23 patients) or directly into a peripheral vein (26 patients). Standard response criteria were used.

RESULTS

There were three partial responses, for an overall response rate of 6% (95% confidence limits, 1%–17%). These responses lasted 1 year, 7+ months, and 5 months, respectively, and occurred in the following dominant sites, respectively: chest wall, lymph node, and lung. Two of these responses were achieved among the seven patients with no prior anthracycline exposure; one response was seen in a patient who had received a single course of a doxorubicin regimen that was withdrawn because of treatment refusal for toxicity. A total of seven patients had stable disease, with a median of 16 weeks (range, 8–30+) from beginning of therapy to disease progression.

The median bisantrene dose per course was 280 mg/m^2, and the median number of infusions per patient was two (range, two to 12). In poor-risk patients, the median wbc count nadir was 2800/mm^3 (range, 900–4900) for the first course, and 2100/mm^3 (range, 500–4000) for all courses. The corresponding figures for the remaining pa-

TABLE 1.—Treatment characteristics

Characteristics	No. of evaluable patients
Total	49
Prior radiotherapy	36
Prior hormone therapy	20
Prior chemotherapy (doxorubicin)	49 (42)
Median No. of cytotoxic drugs (range): 5 (2–14)	
Response to prior chemotherapy (doxorubicin)	
Partial response	28 (10)
No response	17 (30)
Adjuvant only	4 (2)
Dominant sites	
Visceral	29
Skin and soft tissue	15
Bone	5
Platelet count nadir (cells/mm^3)*	
> 75,000	47
⩽ 75,000	2
Other toxic effects*	
Nausea and vomiting (severe)	36 (2)
Hair loss	9
Drug-related fever	5
Hypotension, tachycardia	2
Anaphylactoid reaction	1

* *See* text for dose-limiting toxic effects.

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tients were 2200/mm^3 (range, 800–4200) and 2100/mm^3 (range, 600–3900), respectively. Phlebitis occurred in 19 of 26 patients receiving the drug by peripheral vein and was severe in half of them. Other toxic effects are summarized in table 1.

DISCUSSION

Bisantrene appears to have limited antitumor activity in breast cancer patients after prior doxorubicin therapy. Our findings in such patients are consistent with other reports (1,3). In another series, the results were apparently more positive; there were, however, only two partial responses to bisantrene among the 23 patients whose disease was considered refractory to doxorubicin (4).

In contrast, bisantrene seems to be an active agent against breast cancer in doxorubicin-naive patients. Small numbers of patients preclude definite conclusions, but in this more favorable population we observed two responses in seven patients, whereas Osborne et al (1) reported four responses in 11 patients. Data were even more promising in the M. D. Anderson trial (4) with seven responders to bisantrene among 14 patients previously treated with doxorubicin but whose disease was not refractory to it.

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Phase II Trial of Diaziquone in Advanced Ovarian Carcinoma[1,2]

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For the European Organization for Research on Treatment of Cancer Early Clinical Trials Group

Diaziquone (AZQ) is a quinone derivative that exhibits significant activity against a number of experimental murine tumors (1).

We report a phase II study of AZQ in previously treated patients with advanced ovarian carcinoma.

MATERIAL AND METHODS

Criteria for entry in the study included histologically proven cystadenocarcinoma or undifferentiated epithelial advanced ovarian carcinoma, clinically resistant to conventional therapy; measurable progressive disease within the last 2 months; performance status of 0–2 [World Health Organization (WHO) scale]; expected survival of at least 2 months; and no chemotherapy or radiotherapy during the previous 4 weeks. Normal wbc count, platelet count, bilirubin, and creatinine levels were mandatory.

AZQ was given at doses of 30 mg/m^2 every 4 weeks to patients with good bone marrow reserve, and 25 mg/m^2 every 4 weeks to heavily pretreated patients. Provisions for dose modifications according to myelosuppression have been described previously (2). An adequate trial of AZQ required at least two cycles or rapid progression of disease after one cycle. Response criteria was defined accordng to WHO standards (3).

RESULTS

Twenty-three patients were included in the study; of these, four were eventually excluded, since they did not meet all the inclusion criteria. Eighteen patients were evaluable for toxicity and 17 for response. One early death due to progressive disease (ie, within 28 days after the first course) was not evaluable for toxicity. Patient characteristics and drug toxicity are listed in tables 1 and 2.

One partial response lasting 9 months was observed in a patient with a supraclavicular lymph node and liver metastases. The patient was treated for 5 months and then taken off study due to partial obstruction of the small bowel. The response rate was thus 6% (95% confidence limits, 0.2%–28.7%). Four patients had stable disease lasting from 3 to 6 months, and 12 had progressive disease with a median time to progression of 2 months (range, 0.5–3).

Dose-limiting toxic effects were mainly hematologic (table 2), resulting in one septic death due to leukopenia (wbc count, 0.5 × 10^9/L) and one case of life-threatening gastrointestinal bleeding related to thrombocytopenia (platelet count, 9 × 10^9/L), both during the first course. The latter patient was taken off study.

Grade 1 nausea and vomiting was noted in six patients, and grade 2 in four. No other toxic effects were recorded.

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Median age in yrs (range): 56 (43–70)	
Median performance status (range): 1 (0–2)	
Prior treatment	
Surgery	19
Chemotherapy	
⩽ 3 Drugs	3
> 3 Drugs	16
Cisplatin	16
Radiotherapy	0
AZQ treatment	
Median No. of courses (range): 2 (1–6)	
Initial doses	
25 mg/m^2	11
30 mg/m^2	8

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TABLE 2.—Hematologic toxicity

	Median nadir	Range
1st course		
Wbc count ($\times 10^9$/L)	3.2	0.5-5.2
Platelet count ($\times 10^9$/L)	95	9-275
Hemoglobin (mmol/L)	6.8	5.7-11.9
All courses		
Wbc count ($\times 10^9$/L)	2.7	0.5-5.2
Platelet count ($\times 10^9$/L)	104	9-275
Hemoglobin (mmol/L)	6.6	5.2-11.9

DISCUSSION

In this phase II study of AZQ in heavily pretreated patients with advanced ovarian carcinoma, only one partial response was achieved in 17 evaluable patients. The hematologic toxicity was considerable.

Phase II studies of AZQ have been performed in patients with lymphoma and malignant melanoma (4,5). The response rates and toxicity in these trials equal ours.

Accordingly, AZQ has no substantial activity in this dose and schedule in the treatment of patients with previously treated advanced ovarian carcinoma.

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Parkinsonism Following Iv Administration of High-Dose Metoclopramide Used as an Antiemetic Agent During Cancer Chemotherapy

Clinical trials have demonstrated high-dose iv metoclopramide to be extremely effective in reducing nausea and vomiting induced by cisplatin, one of the most emetogenic cancer chemotherapeutic agents (1,2). Side effects of high-dose metoclopramide, including sedation, restlessness, diarrhea, and constipation, are usually mild (1,2). Acute reversible extrapyramidal reactions have been reported to occur in 2%–30% of patients receiving various high-dose iv metoclopramide regimens (1,2). Tardive dyskinesia has also been observed in patients taking chronic oral metoclopramide (3). In several patients, the symptoms persisted > 1 year following the discontinuation of the drug (3).

Additionally, a symptom complex suggestive of parkinsonism has been described with chronic oral use of metoclopramide (4). We report here two patients receiving high-dose iv metoclopramide, administered to prevent cisplatin-induced emesis, who developed the neurological symptoms of Parkinson's disease.

CASE REPORTS

Patient 1

A 78-year-old female with adenocarcinoma of unknown primary involving the abdominal cavity received metoclopramide (2 mg/kg iv q 3 h × 4) during ip chemotherapy with a cisplatin-containing chemotherapeutic regimen. The patient had no personal or known family history of neurological disease; renal function was normal. Following the first course of therapy (approximately 3 weeks after the initiation of treatment), the patient was noted to have developed a resting tremor, masked facies, and mild bradykinesia. Two additional treatment cycles of metoclopramide were administered. Phenothiazines were not administered during any of the treatment courses. Symptoms increased but did not significantly interfere with normal daily activity. Neurological consultation suggested that the patient had idiopathic Parkinson's disease. Metoclopramide ws discontinued, but specific therapy for parkinsonism was not initiated. The patient's symptoms have gradually improved over a 3-month period.

Patient 2

A 70-year-old female with no prior history of neurological dysfunction received high-dose iv metoclopramide (1 mg/kg q 2 h × 4) along with a cisplatin-based iv regimen for metastatic ovarian carcinoma. Phenothiazines were not included in the antiemetic regimen. Physical examination prior to the institution of therapy demonstrated no neurological abnormalities. Symptoms suggestive of parkinsonism, including resting tremor, rigidity, bradykinesia, and postural instability, developed following three cycles of chemotherapy. Metoclopramide was discontinued because of concern that it was either causing or exacerbating the symptoms of parkinsonism. Neurological symptoms subsequently resolved over several months.

DISCUSSION

To the best of our knowledge, this is the first report of drug-induced parkinsonism from high-dose iv metoclopramide used as an antiemetic agent in cancer chemotherapy. Parkinsonism following oral metoclopramide has been observed to occur as rapidly as 3 weeks following the initiation of treatment but usually requires several months to develop (2,4). Symptoms in reported cases have subsided from 2 weeks to 1 year (median, 4 months) after discontinuation of the agent (2,4). Metoclopramide has been demonstrated to be a centrally acting dopamine antagonist (2). While it was initially believed that this drug did not aggravate the symptoms of idiopathic Parkinson's disease or cause drug-induced parkinsonism, this is clearly not the case (2,4). Patients most susceptible to the development of metoclopramide-induced parkinsonism appear to be elderly females (4). It has been hypothesized that patients developing drug-induced parkinsonism have subclinical idiopathic Parkinson's disease aggravated by the dopaminergic antagonism of the offending agent, which in our patients' situation was metoclopramide (5).

In summary, two elderly patients receiving high-dose iv metoclopramide during cisplatin chemotherapy developed parkinsonism. The occurrence of symptoms suggestive of parkinsonism in patients receiving metoclopramide should suggest a drug-induced syndrome and not idiopathic Parkinson's disease. The recognition of this disease entity is important, since treatment for idiopathic parkinsonism may not be effective, while withdrawal of the offending agent can result in clinical improvement (4).

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Acute Renal Failure and Death Following Sequential Intermediate-Dose Methotrexate and 5-FU: A Possible Aderse Effect Due to Concomitant Indomethacin Administration

Sequential intermediate-dose methotrexate (MTX) and 5-FU have recently been used effectively as cytotoxic therapy for many malignancies. This regimen is usually very well tolerated, with only mild transient toxicity to normal tissues when administered with leucovorin rescue. We report here two patients with severe toxic effects from this regimen, which may have been related to interactions with other concurrent drug administration.

CASE REPORTS

Patient 1

The patient was a 56-year-old-male with a history of hyperuricemia, adult-onset diabetes mellitus, Type IV hyperlipidemia, and renal lithiasis, with liver metastases from adenocarcinoma of unknown primary. Initial treatment with 5-FU, doxorubicin, and mitomycin was unsuccessful. On January 26, 1983, he was noted to have progressive hepatic metastases, with fever due to tumor, and worsening glucose intolerance. Indomethacin (25 mg three times daily) and tolbutamide were added to the allopurinol he was previously taking. On February 9 (BUN, 20 mg/dl; creatinine, 1.1 mg/dl; Hgb, 11 g/dl; and wbc count, 13,000/mm^3 with 79% neutrophils), he received MTX (200 mg/m^2) followed in 1 hour by 5-FU (600 mg/m^2). Twenty-four hours after chemotherapy, he took leucovorin (15 mg orally every 6 hours × 6).

The patient was admitted to the hospital 1 week later because of intractable nausea, vomiting, and diarrhea. He was noted to be acutely ill, with an oral temperature of 37.7°C, dehydration, severe mucositis, tender but nonacute abdomen, and hematest-positive stools. Laboratory results were as follows: BUN, 123 mg/dl; creatinine, 9.7 mg/dl; serum MTX level, 1.3×10^{-6} M; Hgb, 9.5 g/dl; and wbc count, 1100/mm^3 with 87% neutrophils. Urinalysis showed a few rbcs; a moderate number of wbcs; and no crystals, casts, or bacteria. Radiographic evaluations failed to indicate any evidence of pleural or peritoneal effusions or hydronephrosis. The patient was treated iv with leucovorin and appropriate iv fluids. Soon after admission, he became severely granulocytopenic and febrile. Empiric broad-spectrum antibiotics were given when no source of his fever was found. Despite continued antibiotics and aggressive supportive care, he continued to deteriorate and died within 72 hours. An autopsy was refused by the family.

Patient 2

The patient was a 72-year-old, mildly hypertensive male with intermediate-grade adenocarcinoma of the colon metastatic to the liver and peritoneum, who was initially treated with 5-FU, teniposide, and mitomycin on a phase II protocol. He had stable disease for several months. In late November 1983, the diagnosis of superficial thrombophlebitis of the calf was made and indomethacin (25 mg four times daily) was started. This was therapeutically effective and was continued through his admission to the hospital on December 15, 1983. On December 5, 1983 (BUN, 15 mg/dl; creatinine, 1.0 mg/dl; Hgb, 10.3 g/dl; and wbc counts, 7000/mm^3 with 83% neutrophils), he received MTX (200 mg/m^2), followed in 1 hour by 5-FU (600 mg/m^2). Twenty-four hours after chemotherapy, he took leucovorin (15 mg orally every 6 hours × 6).

On December 15, 1983, the patient was admitted to the hospital with a 5-day history of fever, weakness, confusion, and intractable nausea and vomiting. His physical examination revealed confusion, hypotension, axillary temperature of 37.9°C, severe mucositis, and a tender abdomen with guarding. Laboratory results included the following: BUN, 108 mg/dl; creatinine, 4.3 mg/dl; Hgb, 9.7 g/dl; and wbc count, 400/mm^3 with 96% lymphocytes and 4% monocytes. Serum MTX level was 8.1×10^{-8} M. Despite aggressive treatment with fluids, blood, iv leucovorin, and antibiotics, he died about 12 hours after admission. The family refused to permit an autopsy.

DISCUSSION

The lethal toxicity of sequential MTX and 5-FU with leucovorin rescue demonstrated by the two presented cases is remarkable. Numerous reports indicate that this regimen is almost universally well-tolerated, with only rare serious toxicity. Although indomethacin, allopurinol, and MTX have all individually been implicated as causes of renal failure, we believe it is a more likely possibility that the lethal effects of chemotherapy in both presented patients were due to a previously unrecognized adverse drug interaction between indomethacin and intermediate-dose MTX.

Indomethacin-associated acute renal failure is believed to be caused by an inhibition of renal prostaglandin (PGE_2) synthesis (1-4). PGE_2 is a vasodilating agent which also inhibits renal renin release. These actions may decrease renal perfusion, with resultant decreased MTX clearance and increased renal and systemic toxicity. It should be noted that indomethacin does not appear to significantly alter MTX-albumin binding and therefore should not result in enhanced toxicity due to an increase in serum-free MTX.

Renal failure due to MTX alone has frequently been reported and is more common in high-dose administration. MTX is an unlikely cause of renal failure at the doses used in the treatment of our patients, although this toxic effect has been reported with lower doses of MTX without leucovorin rescue (5).

In summary, we believe that the deaths due to toxic effects of chemotherapy, as described above, are secondary to adverse drug interactions. Our postulate is that subclinical renal damage secondary to indomethacin was present at the time of MTX administration. This interfered with MTX excretion and resulted in the severe adverse reactions described. We are therefore recommending that indomethacin not be given concurrently with intermediate- or high-dose MTX unless renal function is tested by creatinine clearance just prior to treatment and excellent hydration is assured.

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N-Methylformamide-Induced Hypophosphatemia[1]

N-Methylformamide (NWF), a polar solvent first introduced in 1953, is undergoing extensive phase II evaluation in the United States (1-3). Such solvents, which include dimethyl sulfoxide and dimethylformamide, not only induce differentiation in human tumor lines, converting neoplastic cells into a more benign phenotype, but also inhibit growth of human tumor xenografts in nude mice (3). Initial clinical trials with daily oral administration had been stopped in 1956 because of severe hepatotoxicity, a reversible dose-limiting effect again described in recent preclinical and phase I studies that employed the following schedules: monthly, iv, daily × 5; every 6 weeks, iv, weekly × 3; and monthly, orally, every other day × 3 (2-4). Other toxic effects have included nausea, vomiting, anorexia, sleeplessness, neuropathy, and flushing. Myelosuppression is uncommon. Although doses up to 1170 mg/m^2 daily have been given, most trials start with 800-1000 mg/m^2 daily for 5 consecutive days, repeated monthly.

[1]Supported by Phase II/III contract 3798 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

In a phase II disease-oriented trial in renal cell carcinoma, five of seven patients developed hypophosphatemia during the first cycle (five of five given 1000 mg/m^2 daily) (table 1). The serum phosphorus concentration rapidly decreased by Day 3 and continued through Day 5, unless oral or iv phosphorus replacement was begun. The median phosphorus nadir was 2.1 mg/dl (range, 1.5-3.0), and occurred on Day 5 (median; range, 3-9). No consistent changes were seen in the serum calcium or albumin levels. Coupled with having the low serum phosphorus level, four patients described diffuse muscular aches and pains, and a "restlessness" often accompanied by an inability to sleep. Such signs could be attributed to hypophosphatemia, since central nervous system abnormalities resulting in generalized anorexia and malaise, weakness, paresthesias, muscle pains, ataxia, seizures, and coma have been described with low phosphorus levels. Patients with muscular aches and pains, restlessness, sleeplessness, and low phosphorus levels were treated with oral phosphorus (250 mg three to four times per day) and soporifics, which seemed to alleviate these symptoms. Symptoms did not recur in three patients

TABLE 1.—Serum levels (initial/nadir) during the first cycle of NMF

Patient No.	Dose of NMF (mg/m^2 x days)	Phosphorus* (mg/dl)	Calcium*,† (mg/dl)	Albumin*,† (g/dl)
1	1000 × 5	3.1/1.5	10.1/9.4	4.8/4.5
2	1000 × 4	3.6/1.7	9.8/9.9	4.9/4.0
3	1000 × 3	4.1/1.9	9.3/7.9	4.0/3.1
4	1000 × 5	3.2/2.1	10.5/9.6	3.2/3.1
5	1000 × 5	3.2/2.1	9.1/9.2	4.2/3.8
6	800 × 5	3.9/2.5	9.4/9.6	4.1/3.9
7	800 × 5	4.2/3.0	9.0/9.4	3.8/3.0

*Normal values: phosphorus, 2.5–4.2; calcium, 9.2–10.8; albumin, 4.0–5.2.
†Levels at time of phosphorus nadir.

given oral phosphorus, 2 g/day, beginning 2 days prior to, and for 3 days after, NMF therapy during Cycles 2 and 3.

The etiology of NMF-induced hypophosphatemia is unclear, but it can be caused by an inadequate oral intake due to malabsorption or vomiting, phosphate depletion through urinary loss in disorders affecting renal tubular phosphate transport or proximal tubular reabsorption, and transcellular shifts resulting in redistribution of phosphate into liver and muscle, ie, following iv administration of glucose and during respiratory alkalosis (5). Perusal of the available literature did not mention clinically significant hypophosphatemia as a possible toxic effect of NMF (6), although hypophosphatemia (levels not defined) was listed in 11 of 32 NMF cycles [table 3, (4)].

NMF-induced hypophosphatemia is under investigation at Memorial Hospital. Physicians should be alerted to this potential problem in phosphate homeostasis when treating patients with NMF, particularly with doses of 1000 mg/m^2.

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Fatal Pneumothorax Following Bleomycin and Other Cytotoxic Drugs

We describe here a case of fatal pneumothorax in a patient with probable pulmonary fibrosis following chemotherapy which had included bleomycin to a total dose of 420 mg in 5 years. At the time of the pneumothoraces, the patient had not received chemotherapy for 4 months and had no known pulmonary metastases. The fatal pneumothoraces are thought to be the result of the pulmonary fibrosis.

A 19-year-old fireman presented in 1976 with rubbery, painless cervical lymphadenopathy and splenomegaly. Nodal biopsy showed Hodgkin's lymphoma of mixed cellularity type. Investigations, including laparotomy and splenectomy, showed stage IV disease involving cervical, mediastinal, and para-aortic lymph nodes, liver, and spleen.

Chemotherapy was begun with mechlorethamine, vinblastine, procarbazine, and prednisolone (table 1). After six courses, there appeared to be a complete clinical remission. An additional four courses were given at 3-month intervals until February 1978, when he relapsed with cervical and right axillary lymphadenopathy. Biopsy then showed lymphocyte-predominant Hodgkin's disease. Second-line chemotherapy with doxorubicin, bleomycin, vincristine, and dacarbazine was started, but the patient refused further treatment after the first course. At that time he had received a total of 60 mg of bleomycin.

In October 1978, he suffered ophthalmic herpes zoster. In January 1979, he developed severe Coombs-positive hemolytic anemia (hemoglobin, 4 g/dl; reticulocyte count, 50%). He accepted treatment with blood transfusions, oral azathioprine (150 mg), and prednisolone (60 mg)

TABLE 1.—Chemotherapy given

Drug	Dates given	Total dose (mg)
Mechlorethamine	1976-1977	200
Vinblastine	1976-1977	200
Procarbazine	1976-1977	21,000
	1979	6300
Doxorubicin	1978	100
Bleomycin	1978	60
	1981-1983	360
Vincristine	1979	12
Chlorambucil	1979	520
Lomustine	1981-1983	1920
Vindesine	1981-1983	72

daily. Hemolysis continued and he then agreed to three courses of vincristine, chlorambucil, procarbazine, and prednisolone with radiotherapy to bulky cervical nodes.

This achieved remission of the hemolytic anemia and partial remission of the lymphadenopathy. Radiotherapy to the axilla, groins, and abdomen controlled the condition until October 1981. Troubled by weight loss and lassitude, he accepted further chemotherapy using bleomycin, lomustine (CCNU), and vincristine, with considerable improvement, although enlarged abdominal lymph nodes remained palpable.

By February 1983, he had received a total of 420 mg of bleomycin and no further courses were given. Routine lung function studies suggested a restrictive defect of lung function [forced expiratory volume in 1 second (FEV1), 2.3 L (predicted, 4.72); forced vital capacity, 2.6 L (predicted, 5.53)]. In March, the chest x-ray showed a slightly raised diaphragm but normal lung markings.

In May 1983, he developed progressive dyspnea, but examination of the chest was normal, apart from tachypnea. Lung function studies now showed FEV1 of 0.9 L; forced vital capacity of 1.05 L; and single-breath transfer factor of 10.5 ml of CO/minute (predicted, 31.9). A chest x-ray still showed normal lung markings. In retrospect, a very small pneumomediastinum has been seen and the significance of this has now become apparent in light of the patient's subsequent clinical course. Ventilation and perfusion isotopic lung scans showed no evidence of pulmonary emboli. "Bleomycin lung" was suspected and he was treated with prednisolone, 40 mg/day. In June 1983, he was admitted to the hospital urgently with severe dyspnea and cyanosis. A right pneumothorax with > 50% collapse of lung and a small left pneumothorax were seen on chest x-ray. Despite chest drains (ultimately in three different sites) with application of negative pressure of 5-10 mm Hg, the right lung would not fully reflate. He died following sudden deterioration when the left lung also collapsed. Unfortunately, permission for autopsy was not obtained and we have no tissue diagnosis.

Pulmonary toxicity of bleomycin, giving rise to interstitial pneumonitis and fibrosis, is well-recognized (1). Spontaneous pneumothorax has been described in "BCNU lung" (carmustine) (2) and during combination chemotherapy (3,4), usually during regression of pulmonary tumor deposits. This patient had stopped chemotherapy 4 months before and had no radiologically visible lung metastases. He had been developing features suggestive of "bleomycin lung," although chlorambucil, procarbazine, and lomustine (5), as well as radiotherapy to axilla and neck, may have contributed. The total dose the left upper lobe may have received was 2000 rad and the cumulative dose to the right upper lobe was 1000 rad over 1 year. Fatal pneumothorax now appears to be part of the spectrum of pulmonary complications from bleomycin and other cytotoxic drugs, even after the cessation of treatment.

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NCI–INSERM Cooperation in Basic and Clinical Cancer Research

The US National Cancer Institute (NCI) and the French Institut National de la Santé et de la Recherche Médicale (INSERM) jointly sponsor an agreement to promote cooperation in basic and clinical cancer research. Limited funds are now available for this program to provide travel expenses and subsistence allowance for US scientists to work in France for periods of up to 1 year on appropriate collaborative research projects with French colleagues. In addition, similar funds are available to pay the living expenses for French scientists to work in the United States on collaborative cancer research projects with American colleagues. Each request for support will be reviewed for scientific merit by the American and French program committees. The deadline for applications for this year is *April 1, 1985.*

For further information, contact: Office of International Affairs, National Cancer Institute, Bldg 31, Rm 4B55, Bethesda, MD 20205; or Dr. Jean Paul Levy, Institut National de la Santé et de la Recherche Médicale, 101 rue de Tolbiac, 75654 Paris CEDEX 13, France.

NCI/US–NCI/Milan Cooperation in Basic and Clinical Cancer Research

The US National Cancer Institute (NCI) and the National Cancer Institute of Milan jointly sponsor an agreement to promote cooperation in basic and clinical cancer research. Limited funds are now available for this program to provide travel expenses and subsistence allowance for US scientists to work in Italy for periods of up to 1 year on appropriate collaborative research projects with Italian colleagues. In addition, similar funds are available to pay the living expenses for Italian scientists to work in the United States on collaborative cancer research projects with American colleagues. Each request for support will be reviewed for scientific merit by the American and Italian program committees. The deadline for applications for this year is *April 1, 1985.*

For further information, contact: Office of International Affairs, National Cancer Institute, Bldg 31, Rm 4B55, Bethesda, MD 20205; or Dr. Umberto Veronesi, Director, Istituto Nazionale Tumori, Via Venezian, 1, Milan, Italy.

11th Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders: Medical, Surgical and Radiotherapeutic Aspects

Baltimore, Maryland, April 11–12, 1985

The Oncology Center of The Johns Hopkins School of Medicine will present this symposium to provide updates on recent significant developments in oncology, as well as state-of-the-art reviews of the management of common tumors. The program will include discussions of non-Hodgkin's lymphoma, leukemia, brain tumors, and soft tissue sarcomas; the importance of innovations in supportive care will be recognized in reviews of nutritional support and antibiotic and antiviral therapies.

This 2-day program is rated for 16 CME Category I credit hours by the AMA. The fee is $225, which includes social activities.

For further information, contact: Program Coordinator, Continuing Education, Turner Auditorium, Rm 22, 720 Rutland Ave, Baltimore, MD 21205 (telephone: 301-955-6046).

Basic Biostatistics and Epidemiology: Two Short Courses

Bethesda, Maryland, April 29–May 3, 1985

The New England Epidemiology Institute will present two consecutive short courses at the Linden Hill Hotel in Bethesda. "Basic Biostatistics" (April 29–30) will be taught by Drs. William Louv and Harland Austin and will present an overview for people who wish to become conversant with the fundamental concepts of biostatistics. "Principles of Epidemiology" (May 1–3) will be taught by Drs. Kenneth Rothman and Stephan Lanes; it will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and

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Registrants may receive continuing medical education credits (AMA Category 1) and/or certification maintenance credits from the American Board of Industrial Hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, P.O. Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Diet and Human Carcinogenesis

Aarhus, Denmark, June 19–21, 1985

The aim of this Third Annual Symposium of the European Organization for Cooperation in Cancer Prevention Studies (ECP) is to present to the scientific and general communities a synopsis of the most recent knowledge regarding the epidemiological and biological aspects of diet as related to human carcinogenesis. The following topics will be covered: causal and protective agents in human carcinogenesis; diet and breast cancer; diet and digestive tract cancers; diet, cancer, and other diseases; role and feasibility of prospective and intervention studies on diet and cancer; and problems involved in dietary counseling.

The symposium will also include: a panel discussion on the dietary recommendations prepared in a joint IUNS (International Union of Nutritional Sciences) and ECP workshop; a seminar on the value of different methodological approaches in epidemiologic studies on diet and cancer; and a poster session. Participants are invited to present poster papers. Registered participants should send abstracts of their papers for review by the scientific committee no later than *April 15, 1985*. Abstracts should not exceed one 21 × 29.7-cm page (including authors' names, affiliations, and text).

For further information, contact: Mr. J. Geboers, Lic. Sc., Secretary-General, 3rd ECP Symposium, c/o Division of Epidemiology, Sint-Rafaël University Hospital, Capucijnenvoer 33, B-3000 Leuven, Belgium.

Fifth Annual Epidemiology Summer Program

Amherst, Massachusetts, July 28–August 16, 1985

The University of Massachusetts will host a 3-week summer program in epidemiology which will include both methodologic and substantive courses and will offer classes for participants seeking an introduction to modern epidemiologic concepts as well as those desiring a review of recent developments in epidemiologic thinking. Thirteen courses will be offered; invited faculty include excellent teachers and prominent researchers from leading universities. Registrants may receive credits toward graduate degrees, continuing medical education, or certification maintenance in industrial hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, P.O. Box 57, Chestnut Hill, MA 02167 (telephone 617-734-9100).

Joint Conference of the 17th International Leukocyte Culture Conference and the 22nd National Meeting of the Reticuloendothelial Society

Ithaca, New York, August 3–9, 1985

This joint conference will be held on the campus of Cornell University. Scientific sessions will consist of symposia, poster sessions, and poster discussion workshops. Symposium topics include oncogenesis and cell growth regulation in normal and neoplastic cells, development and differentiation of T lymphocytes, neuroendocrine-immune interactions, leukocyte biology of natural host defense mechanisms, and cell biology of natural host defense mechanisms. Financial awards will be given for the best student research papers, for the best young investigator's research paper, and for student travel.

For further information, contact: RES/LCC Conference Office, c/o Dr. Sherwood M. Reichard, Medical College of Georgia, Augusta, GA 30912 (telephone: 404-828-2601).

Fourth World Conference on Lung Cancer

Toronto, Canada, August 25–30, 1985

Sponsored by the International Association for the Study on Lung Cancer and following in the tradi-

tion of the three previous world conferences on lung cancer in 1978, 1980, and 1982, the Fourth World Conference will emphasize a multidisciplinary approach to lung cancer. It will highlight current knowledge in biology, prevention, diagnosis, treatment, and supportive care. A full schedule of plenary sessions, mini-symposia, and selected abstracts presented as posters or talks will allow exploration of new and innovative ideas.

The conference has been designated as a continuing medical education activity for 24 credit hours in Category 1 of the Physicians' Recognition Award of the American Medical Association.

For further information, contact: Fourth World Conference on Lung Cancer, Secretariat Office, 342 MacLaren St, Ottawa, Ontario, Canada K2P OM6.

Second International Symposium on Detection and Treatment of Minimal Residual Disease in Acute Leukemia

Rotterdam, The Netherlands, November 6–8, 1985

This symposium is focused on the detection and treatment of subclinical disease in acute leukemia, ie, the small numbers of neoplastic cells which frequently persist following successful remission induction therapy and cause relapse. The organizers are Drs. Anton Hagenbeek and Bob Löwenberg; topics will include new methods to detect residual leukemia, the value of new chemotherapy programs, and recent accomplishments in clinical bone marrow transplantation.

Abstracts for free poster sessions must be received before *August 1, 1985.*

For further information and abstract forms, contact: J. W. van der Velden, MD, c/o Organizing Committee Leukemia Symposium, The Dr. Daniel den Hoed Cancer Center, P.O. Box 5201, 3008 AE Rotterdam, The Netherlands.

Award for Outstanding Research in the Field of Medical Oncology

The European Society for Medical Oncology (ESMO) invites nominations for an award of $10,000 for research in clinical cancer chemotherapy. The purpose of the award is to recognize outstanding achievement in cancer chemotherapy research and to provide financial assistance to candidates working in this field.

The award is made possible by an educational grant from Bristol-Myers Co, New York, NY, and is open to European medical oncologists under the age of 35 years who are working in the field of clinical cancer chemotherapy. Selection of the award recipient will be made by the ESMO Award Selection Committee at the end of April 1985; the award will be presented in Stockholm, Sweden, in June 1985, at the Third ECCO Meeting.

For further information, contact: Secretariat ESMO, Mme. F. Fein, Centre Antoine-Lascassagne, 36 Voie Romaine, 06054 Nice Cedex, France (telephone: 93-81-71-33, ext 2514).

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Cancer Treatment Symposia: Proceedings of the Workshop on Mitoxantrone Cardiotoxicity and the Workshop on Clinical Evaluation of Anthracycline Cardiotoxicity

This volume containing the proceedings of two important workshops on cardiotoxicity related to the administration of mitoxantrone and doxorubicin is now available. These workshops were sponsored by the Division of Cancer Treatment of the National Cancer Institute.

Held at the National Institutes of Health on July 13, 1983, the Workshop on Mitoxantrone Cardiotoxicity focused on reports of the clinical testing of mitoxantrone, an agent that has shown promise for the treatment of hematologic malignancies and breast cancer. The objectives of the workshop were to assess the preclinical and clinical evidence regarding the cardiotoxicity of mitoxantrone and to characterize its clinical features; to estimate more precisely the incidence of cardiotoxicity attributable to this drug; and to discuss methods of further defining its cardiotoxic potential, with specific emphasis on risk factors, monitoring techniques, and comparative evaluation of animal models.

The Workshop on Clinical Evaluation of Anthracycline Cardiotoxicity was held at the National Institutes of Health and The Ohio State University College of Medicine on October 3–5, 1983. The chief aim of the workshop was to promote discussion of the most important issues concerning cardiac monitoring and to outline an effective and relatively efficient strategy for the generation of reliable data on comparative cardiotoxicity. Because the anthracycline doxorubicin is one of the most effective chemotherapeutic agents available, much clinical research has been directed to methods of lessening its toxicity to the heart.

Copies of this volume of *Cancer Treatment Symposia* (Volume 3, 1984) are available for $4.75 from the US Government Printing Office. To order, use the form below. Make checks payable to the Superintendent of Documents and mail to the address on the order blank.

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